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AFSTI
ASSOCIATION OF FOOD SCIENTISTS
& TECHNOLOGISTS (INDIA)

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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) MYSORE - 570 013

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* Advancement of all the aspects of Science and Technology relating to production, processing and distribution of food, with the ultimate objective to serve humanity through better food.
* Promotion of research, development and training in the science, Technology and Engineering of Food.
* To provide a forum for exchange, discussion and dissemination of knowledge and current developments, especially among Food Scientists and Technologists as well as the Public and Society at large.

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* Holding symposia/conventions on different aspects of Food Science, Technology and Engineering.
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REVIEW

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Role of Acidulants in Food Industry

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Acidulants namely acetic, adipic, citric, fumaric, lactic, malic, phosphoric and tartaric acids, and glucono-delta-lactone are commonly used as food additives in processed foods and beverages to not only impart sour taste but also to adjust the pH, enhance and modify the flavours and sweetness of sugars, perform leavening functions in baked goods, control gel formation and maintain viscosity of confections and gelatine desserts, etc. The intensity of sourness and ability to reduce pH vary among the organic group of acidulants in the decreasing order of fumaric > tartaric > malic > acetic > citric > lactic > gluconic acids. The present review attempts to appraise the chemistry, functional properties, food applications, and food regulations and toxicology of individual acidulant in context with newer developments in food and beverage processing. The criteria for selection of a specific acidulant for a specific food application are also discussed for the benefit of the food and beverage processors.

Keywords : Acidulants, Additives, Organic and inorganic chemistry, Food and beverage applications, Food regulations, Toxicology, Selection criteria.

A food is subjected to various treatments before it is made palatable and taste is an important factor for a food to be accepted or rejected. If a food lacks the desired taste, it will be rejected regardless of its nutritive value. There are four primary tastes-sweet, sour (acidic), salty, and bitter that human palate perceives when the food is eaten. The sour or acidic taste of a food is attributed to the acidic components naturally present in the food. Many processed foods and beverages, however, require addition of acids to impart characteristic taste to the final product. These are commonly called acidulants. Some of the commonly used acidulants include acetic, adipic, citric, fumaric, lactic, malic, phosphoric and tartaric acids and glucono-delta-lactone, which find specific applications within the food industry (Butters 1986).

Many of these acids occur widely in natural foods of both plant and animal origins arising therein during the course of their metabolic activities. Citric, malic and fumaric acids are involved in anaerobic respiration or Krebs cycle. Lactic acid arises at the end of anaerobic respiration through glycolysis pathway, whereas phosphoric acid is involved in energy transfer systems in the living cell. These acids impart characteristic acidic taste to the natural foods in which they occur, many a time in combination. Fruits like orange, kinnow, lemon, galgal, pineapple, apple, plum, grape, mango, apricot are strongly acidic. The taste and flavour attributes of these and many other fruits are associated with the "sugar : acid" ratio in them. This ratio changes with maturity of the fruit and serves as maturity index as well. Most vegetables and fresh meats are, however, mildly acidic. Tomato is strongly acidic. The fermented foods also acquire acidity due to acetic or lactic acids formed during the course of fermentation, e.g. yoghurt, sauerkraut, pickles, vinegar, etc. These acids are therefore, not alien as food additives commonly used in food industry to impart a variety of functions. Table 1 lists some of the important food acids and the main foods in which these occur or are added during processing (Butters 1986).

Since the composition of similar types of raw materials, e.g., tomatoes, differs due to varietal and environmental factors, it becomes mandatory to standardise the food products by adding suitable additives during the preservation and processing steps exercised in food industry. Acidulants are such food additives extensively used to provide benefits of many of their natural functions for uniform taste acceptability of processed food products. Without the use of acidulants, many of the familiar soft drinks we know would not exist. The acidulant of choice for a particular food product provides desired sour or tart taste and/or adjusts the sugar: acid ratio for balanced flavour of that food product. To develop the flavour of the food to its fullest potential, it is desirable to fine-tune this ratio. Some of the acids have synergistic effect on the flavour of a food because of their own characteristic flavour and the degree of tartness they impart. For example, phosphoric acid goes well with cola flavours, whereas tartaric acid is compatible with grape flavour (Dziezak 1990).

The sour taste response imparted to a food is attributed to the hydrogen (H^+) or hydronium (H_3O^+) ions. However, sourness is reported to be independently influenced by concentration, pH and anions species of the acid. The acidic taste offered by these food acids is, therefore, not the same. There are subtle differences in their taste characteristics as observed by the trained taste panel (Table 2). The acidulants at equal concentration, vary in the degree or intensity of sourness and is in the decreasing order of fumaric > tartaric > malic > acetic > citric > lactic > gluconic acids (Watine 1995). These differences may be attributed to the effect of free anions associated with different acidulants, on the palate. Also, the level of perceivable sourness or tartness may vary in different food products because of differences in overall flavour of the food products in question. Hence, it will not be appropriate to substitute one food acid for another on equal concentration basis. The replacement percentages as shown in Table 3 using anhydrous citric acid as equal to 100% sourness, may be used as a rough guide.

This shows that compared to citric and malic acids, less of phosphoric or fumaric acid would be required to attain a particular titrable acidity or to lower the pH to a given level. However, with the emerging demands for exotic tastes, applications of food acids mix has been envisaged by the food

TABLE 1. MAIN FOODS IN WHICH ACIDULANTS OCCUR OR ADDED TO

Acid	Main food
Acetic acid	Pickles, sauces, relishes, fermented vegetables and fruits, vinegar, wheat bread, cheeses and creams, apple juice, grapefruit juice.
Adipic acid	Beet juice, guava, papaya, raspberry, porkfat, dairy foods, gelatine and desserts, puddings, beverages, jam and jellies, snack foods, condiments.
Citric acid	Oranges, lemons, grapefruit, black-currants, gooseberries, pineapple, raspberries, strawberries.
Fumaric acid	Confectionery, powdered gelatines, desserts, cheese cake, jams and jellies.
Glucono-delta-lactone	Cured meats, frankfurters, salami, sausages, dessert mixes, bakery mixes, processed cheese, fish products, spice preparations.
Lactic acid	Fresh meat, yoghurt, cheese, bread, pickles, sauces, relishes, fermented foods, buttermilk, wines, beer.
Malic acid	Watermelon, plum, apple, cherry, peach, pear, grape, gooseberry, pineapple.
Phosphoric acid	Cola beverages, jams and jellies, bread dough, cake, flour.
Tartaric acid	Grapes, tamarind, pineapple, mulberries, gherkins, wines.

TABLE 2. TASTE CHARACTERISTICS OF FOOD ACIDS

Acid	Taste characteristic
Acetic acid	Strong volatile acid, vinegary smell and astringent taste.
Adipic acid	Sour tasting but lacking tang, chalky note and lingering
Citric acid	Sharp, clean sour taste with little persistence on palate.
Fumaric acid	Strong metallic acidic taste, very lingering on palate.
Glucono-delta-lactone	Weak acidic taste.
Lactic acid	Mild acidic taste but lingering on palate.
Malic acid	Strong but smooth acidic taste, persists longer on palate.
Phosphoric acid	Harsh biting flat acidic taste, lingering.
Tartaric acid	Sharp and bitter acidic taste of short duration.

TABLE 3. SUBSTITUTION OF ONE FOOD ACID FOR ANOTHER

Acid	Percentage
Citric	100
Phosphoric (85%)	50-60
Fumaric	67-72
Malic (fruit flavours)	78-83
Tartaric	80-85
Malic (citrus flavours)	89-94
Adipic	110-115

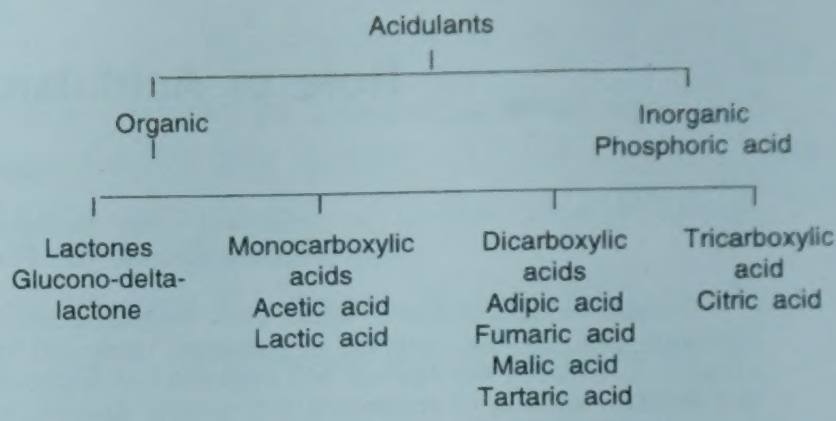

Fig. 1. Classification of acidulants

technologists. The use of co-granulated citric, malic and fumaric acids in appropriate combination is reported to allow for desired variation in tartness or flavour profile of food products (Jindra 1989).

Besides providing tartness or sourness to beverages and many other foods, some of the other many functions of food acids include: pH adjustment, acting as preservative through pH reduction, increasing the effectiveness of preservatives such as benzoates by lowering the pH, enhancing and modifying flavours, modifying the sweetness of sugars and other sweeteners, increasing the thirst-quenching effect of beverages by stimulating the flow of saliva in the mouth, chelate trace metal ions, acting as synergist to antioxidants, buffering beverages when used in the form of buffering salts, leavening functions in baked goods, control of gel formation and maintenance of viscosity in confections and gelatine desserts, texture development in dairy products, etc (Butters 1986; Dziezak 1990)

These acidulatns can be broadly classified into two groups, namely organic and inorganic (Fig.1). Phosphoric acid is the only inorganic acid commonly used in food industry. However, a large number of organic acids are now being marketed as acidulants for a variety of food products (Maga and Tu 1995).

This review encompasses the chemistry, functional properties and food applications of those acidulants that are primarily used to impart sour or tart taste, adjust pH or modify the flavour of a food product.

Acetic Acid

Chemistry : Acetic acid or ethanoic acid or methane carboxylic acid is a monocarboxylic acid with a molecular formula, $C_2H_4O_2$ and formula weight of 60.06. The apparent molecular weight of acetic acid varies with temperature and presence of associating substances. Acetic acid containing less than one percent water is called glacial acetic acid. Structurally, it is represented as (Kirk-Othmer 1995):

$$CH_3 - \overset{\overset{O}{\|}}{C} - OH$$

Occurrence : It forms central part of all biological energy pathways. Humans exude about 70 mg acetic acid per day. It occurs widely in fresh apple, apple juice, grapefruit juice,

canned mangoes, capsicum, cooked or boiled potatoes, vinegar, wheat bread, cheeses and cream, cooked rice, fermented fruits and vegetables. Traces of acetic acid are found in ocean water, oilfield brines, rain and in many plant and animal fluids (Butters 1986).

Preparation : Fermentation of fruits and vegetables juices yields 2-12% acid solution known as vinegar. Different kinds of vinegar can be prepared with distinct flavour profiles attributable to the acid content and various flavour components. Any sugar-containing juice or fluid can be converted into dilute acetic acid by bacterial or fermentation processes. Commercially, acetic acid is produced by oxidation of hydrocarbons e.g. butane oxidation or by methanol carbonylation. However, the acid thus prepared is not permitted for food uses (Maga and Tu 1995).

Properties : Acetic acid is a colourless, water-like liquid having piercingly sharp vinegary odour and a burning taste. It is miscible with water and alcohol. It has acidic taste, when diluted with water. Glacial acetic acid is hygroscopic. Acetic acid has boiling point at 117.87°C, melting point -8.5°C, density (20°C) 1.04928 g/ml, refractive index (nD^{25}) 1.36965, viscosity (25°C) 10.97 mPa.s, ionisation constant (25°C) 1.76 $\times 10^{-5}$, and pka value of 4.75 (Kirk-Othmer 1995; Burdock 1997).

Applications

It is widely used as acidifier, pH control agent, curing/pickling agent, flavouring agent and flavour enhancer. Use of acetic acid as vinegar in foodstuffs and beverages dates back to ancient times. Acetic acid is now commonly added to non-alcoholic beverages (39 ppm), ice cream (32 ppm), candy (52 ppm), baked goods (60 ppm), condiments (5900 ppm), processed cheese and preparations (40,000 ppm). Its usage is limited by good manufacturing practice (GMP) in gravies and sauces, meat products, salad dressings, mayonnaise, canned tomatoes, asparagus, baby foods, sardines, mackerel, and pickled cucumber, onions, boillons and consommes, edible fungi etc (Butters 1986; Ash and Ash 1995).

Food regulations and toxicology : Dilute acetic acid is approved as Generally Recognised As Safe (GRAS) material for food use. Food and Agriculture Organsisation (FAO) has set no limits on its acceptable daily intake (ADI) for humans. Pure acetic acid is moderately toxic by ingestion and inhalation. Glacial acetic acid causes serious gastric difficulties if swallowed. LD_{50} (oral) for rats is 3310 mg/kg body weight. The United States of America (USA) occupation standard for air in workplaces is a time-weighted average of 10 ppm acetic acid (Maga and Tu 1995; Burdock 1977).

Adipic Acid

Chemistry : Adipic acid (1,4-butanedicarboxylic acid) is a dibasic organic acid having a molecular formula $C_6H_{10}O_4$ and a molecular weight of 146.14. The structural formula of the acid is given in Fig. 2 (Kirk-Othmer 1995).

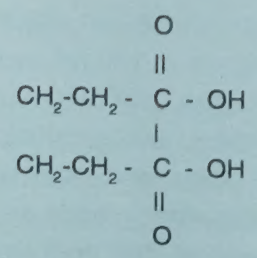

Fig. 2. Structural formula of adipic acid

Occurrence : Adipic acid occurs naturally in beet juice, guava, papaya, raspberry, pork-fat etc.

Preparation : It is commercially produced from cyclohexane by two step oxidation. First, cyclohexane is air-oxidised to cyclohexanone and cyclohexanol, the mixture of which is then oxidised with nitric acid to yield adipic acid. It is then refined for food use. Its worldwide production is estimated to be several million tonnes. But, majority of the adipic acid is used in the production of nylon and other synthetic fibres (Maga and Tu 1995; Burdock 1997).

Properties : Adipic acid is a pure, white crystalline substance. It is odourless with a sour taste. It is sparingly soluble in cold water (1.4 g/100 ml at 20°C) but soluble in hot water (160 g/100 ml at 100°C), and very soluble in methanol and ethanol. It is non-hygroscopic. The pure acid melts at 152°C. Foodgrade acid, however, melts at between 151.5 and 154°C. It boils at 337.5°C. It has ionisation constants (25°C) $K_1 = 3.71 \times 10^{-5}$ and $K_2 = 3.87 \times 10^{-6}$, pKa values of 4.43 and 5.41, respectively (Maga and Tu 1995; Burdock 1997).

Applications : In food industry, adipic acid is generally used as an acidulant, flavouring agent, leavening and pH control agent, and gelling agent. It is added to those food products having delicate flavour and where addition of tang to the flavour is considered undesirable. Its aqueous solutions have the lowest acidity of any of the common food acids. The pH of its solutions (0.5 to 2.4 g/100ml) varies from less than half a unit. Hence, it can be used as a buffering agent to maintain acidities within the range of 2.5 to 3.0. This is highly desirable in certain foods, yet the pH is low enough to inhibit the browning of most fruits and other foodstuffs. In grape-flavoured products, adipic acid adds a lingering supplementary flavour. It performs excellently in promoting rapid setting of gelatine containing desserts. It produces the desired tartness at the optimum gelling pH of 3.0. Due to its buffering action, adipic acid also protects the gelatine desserts against quality loss due to variation in hardness and pH of water. The gelatine powders containing on an average 3.3% adipic acid, do not need any addition of buffer. Baked goods made with adipic acid as the leavening agent are superior in flavour, texture and volume. The use of adipic acid is reported to improve the melting characteristics and texture of processed cheese and cheese spreads. It improves the whipping quality of food products containing egg white. It induces the gel formation in imitation jams and jellies. It helps to extend the shelf life of dry mixes and baking powders, because of its extremely low rate of moisture pick up (Dziezak 1990; Maga and Tu 1995; Ash and Ash 1995).

The food products in which adipic acid is added to perform one of the above functions include, baked goods, bakery powders, beverages, powdered concentrates for fruit flavoured drinks, condiments, dairy products, cheese analogues, gelatine candies, jam, jellies and marmalade, gravies, meat products, instant puddings, snack foods and flavouring extracts. The recommended levels of this acid that can be added to different foodstuffs are: baked goods (0.05%), non-alcoholic beverages (0.005%), condiments (5%), dairy products (0.45%), frozen dairy desserts (0.0004%), gelatines and puddings (0.55%), gravies (0.1%), meat products (0.3%), snack foods (1.3%), others (0.02%) (Ash and Ash 1995).

Food regulations and toxicology : Foodgrade adipic acid should have an assay of 99.6% minimum with a heavy metal content of not more than 10 ppm. The standard methods for the analysis of foodgrade adipic acid are given in the Food Chemicals Codex. Adipic acid has been accorded GRAS status for use as a direct ingredient in foodstuffs. It has been approved by US FDA, U.K., Japan and is listed with EEC. It is relatively non-toxic and is excreted essentially unmatabolised in human urine. The ADI is 0-5 mg/kg. LD_{50} (oral) in mouse, it is 1900 mg/kg body weight (Maga and Tu 1995; Burdock 1997).

Citric Acid

Chemistry : Citric acid (2-hydroxy -1, 2, 3- propane-tricarboxylic acid) is a tribasic acid having a molecular formula $C_6H_8O_7$ and molecular weight 192.43 (Anhydrous). The structural formula of citric acid is given in Fig. 3 (Kirk-Othmer 1995).

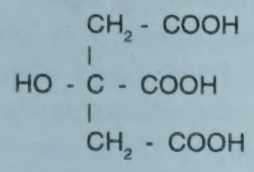

Fig. 3. Structural formula of citric acid

Occurrence : Citric acid is a natural constituent and a common metabolite of plants and animals. It is widely distributed in the tissues and fluids of both plants and animals. It is abundantly found in fruits of the citrus family such as lemons and limes (4-8%) grapefruit (1.2-2.1%), oranges (0.6-1.0%). It also occurs in black currants (1.5-3.0%), strawberries (0.6-0.8%), gooseberries (1.0%), raspberries (1.0-1.3%), apple (0.008%), pineapple, tamarind, potatoes (0.3-0.5%), tomatoes (0.05-1.1%), peas (0.05%), eggplant (0.01%), etc. Its presence in fruits and vegetables influences the taste and flavour characteristics of these commodities (Butters 1986; Maga and Tu 1995).

Preparation : Citric acid, traditionally, was extracted from the juices of lemons and limes and from the pineapple processing wastes. This has since been replaced with the more economical processes of fermentation using different organisms and techniques. The surface culture process and submerged culture process using different strains of *Aspergillus niger,* are the processes commonly employed to manufacture citric acid on commercial scale. The submerged culture process of fermentation using select species of yeast e.g. *Candida guilliermondii,* required shorter fermentation times than the fermentation process using *A. niger.* Citric acid thus produced is generally recovered from the ferments by first separating the microorganisms by filtration or centrifugation, and then precipitating the citrate ions as the insoluble calcium salt. Calcium citrate is filtered out and treated with sulphuric acid to convert the calcium citrate into insoluble calcium sulphate and citric acid (Butters 1986; Maga and Tu 1995).

Properties : Citric acid is a colourless, odourless, crystalline solid with a strong acidic tart taste. It is very soluble in water and alcohol. Solubility very much depends on temperature. It is deliquescent in moist air. It is optically inactive. It has a melting point at 153°C (Anhydrous) and 135-153°C (Hydrous), specific gravity (20-40°C) 1.665 g/ml, (Anhydrous), 1.542 (Monohydrate), ionisation constants (20°C) $K_1 = 7.10 \times 10^{-4}$, $K_2 = 1.68 \times 10^{-5}$, $K_3 = 6.4 \times 10^{-7}$ with corresponding pKa values 3.14, 4.77, 6.39, respectively (Kirk-Othmer 1995; Burdock 1997).

Applications : Food industry uses citric acid for its functions as an acidulant, flavouring agent, flavour modifier or enhancer, curing accelerator, dispersing agent, sequestrant and as a synergist for antioxidants (Butters 1986; Ash and Ash 1995; Anon 1995/96). It is the acidulant of choice for the food manufacturer because of its natural tang, "burst" of tartness and its rapid solubility. It is generally used in solid form but use of liquid citric acid in food industry has several advantages as follows :

a) ingredients may be added simultaneously rather than sequentially during processing, (b) standardising the citric acid solution at 50% avoids potential human error, when granular citric acid is dissolved prior to use, (c) ease in handling as the solution is pumped directly into the production line, minimising the risk of spillage and contamination, (d) losses of the powdered acid in seams and folds of packaging are eliminated, and (e) labour and handling costs are reduced by the elimination of the need for on site boilers to pre-dissolve the acid crystals.

The only disadvantage is the high cost of transportation of liquid citric acid. However, a spray granulation process which builds up particles layer by layer, producing ultraspherical particles of citric acid has been developed. The crystal size (200 to > 300 μm) and composition are designed to suit food industry requirements. Such ultraspherical crystals can be handled by fluidised bulk systems, reducing packaging and labour costs. Co-granulation of two or more acids e.g. citric, malic or fumaric, may allow for variations in tartness of flavour profiles (Anon 1995/96).

Citric acid as solid or liquid is added to a variety of food products and beverages to impart one or more of the above functions (Dziezak 1990; Ash and Ash 1995; Maga and Tu 1995).

Beverages : There are four different types of beverages being marketed; carbonated, non-carbonated, dry and low-calorie beverages. All require the addition of acidulants to impart tart taste, provide flavour balance, modify the sweetness of sugar and other sweeteners, and extend product shelf life

by lowering the pH and by improving the effectiveness of antimicrobial agents like salts of sorbic and benzoic acids. In carbonated beverages, citric acid provides tartness and compliments fruit and berry flavours. The amount of acid used depends upon the flavour of the product, consumer preference and taste evaluation. Most fruit flavoured carbonated beverages contain citric acid in the range of 0.1-0.25%. To the non-carbonated fruit drinks, which are a mixture of water, fruit juice, sugar, acid, colour, and flavour, citric acid is added to adjust the pH of the drink for uniform acidity. The amount used varies between 0.25-0.4%. The soft drink dry mixes which are mixture of sugar, acid, flavour, colour, buffers, vitamins, fruit pulp, clouding, bodying and free-flowing agents also contain citric acid varying from 1.5 to 5% depending upon the flavour used.

Preserves : In the manufacture of jams, jellies, marmalade and other preserves, citric acid is used to impart tartness and to adjust the pH of the product. The pH may range from 3.0 to 3.4 depending upon the type of pectin used. In the preparation of these products, all the ingredients other than the acid are cooked to the proper soluble solid contents (°Brix). Then, citric acid is added as a 50% solution to ensure good distribution throughout the batch.

Confections : In confectionery industry, citric acid is used in flavours to the level of 0.5 to 1.0% in regular hard candy and in some preparations, it can be as much as 2%. Citric acid imparts tartness or tang, which is compatible with majority of the hard candy flavours. In order to minimize sucrose inversion, the acid is added along with the colour and flavour to the molten candy glass after the cook. Inversion of sucrose to glucose and fructose, renders the candy hygroscopic and susceptible to crystallisation. Where candy manufacture employs depositing systems, solution of buffered citric acid is preferred. Buffered acid is made with a ratio of three parts citric acid to one part sodium citrate. Citric acid is also used in soft candies of gummy or jelly types which are stabilised with pectin. Buffered citric acid is generally used in many fruit-flavoured confections containing high-methoxyl pectins. The acid adjusts the pH to 2.5 to 3 which is optimum for gel formulation and the candy sets to a firm texture. In starch-gelled candies, the acid modifies viscosity, promotes clarity and accentuates flavour. The use of encapsulated citric acid and flavours in chewing gum composition may prolong sourness flavour and juiciness of the product.

Citric acid is also used in sherbets, water ices, gelatine desserts, wine as flavour adjunct and to control pH and acidity of the product. In gelatine desserts, citric acid solubilizes rapidly and imparts tart taste, whereas sodium citrate acts as buffering agent, increasing pH for optimum gel strength.

The recommended usage levels of citric acid vary from product to product, for example ; non-alcoholic beverages (2500 ppm), ice cream and ices (1600 ppm), candy (4300 ppm), baked goods (1200 ppm), chewinggum (3600 ppm), processed cheese (40000 ppm), canned baby foods (15000 ppm), processed cereal-based foods for infants and children (25000 ppm on dry weight basis), cocoa powder and dry cocoa-sugar mixtures (5000 ppm) (Burdock 1997).

However, there are no limits for acceptable daily intake for humans.

Food regulations and toxicology : The food applications of citric acid are governed by Food Chemicals Codex specifications. It should conform to the pyridine-acetic anhydride tests for identity and should not contain less than 99.5% citric acid (anhydrous), ash 0.05%, heavy metals 0.001% (maximum). The Bureau of Indian Standards (BIS 1970) demands that foodgrade citric acid (monohydrate) should comply with the following requirements.

(a) it should be colourless, translucent crystals or a white granular to fine crystalline powder, (b) it should be odourless with a strong acid taste, (c) it should be freely soluble in water, ethyl alcohol and sparingly soluble in ether, it shall form a clear solution in water, (d) the material should not contain less than 99.5% citric acid monohydrate by weight, (e) it should not contain more than 10 ppm halides, 10 ppm heavy metals. 1 ppm arsenic and 8.8% water by weight and (f) it should pass the oxalate test.

Citric acid is, however, universally accepted as a safe food ingredient. US FDA lists this acid as a multiple purpose GRAS food substance. It is also approved by the joint FAO/ WHO Expert Committee on food additives for use in foods without limitations. The Fruit Products Order (1955) permits it to be added in fruit products as per good manufacturing practice. It is, however, mildly toxic by ingestion and is severe eye and moderately skin irritant. $LD_{x\ 50}$ (oral) in rats is 67,300 mg/kg body weight (Maga and Tu 1995; Burdock 1997).

Fumaric Acid

Chemistry : Fumaric acid (trans 1, 2-ethylenedicarboxylic acid) or 2-butenedioic acid is an unsaturated dicarboxylic acid having a molecular formula $C_4H_4O_4$ and molecular weight 116.07. Its structural formula is given in Fig. 4 (Kirk-Othmer 1995).

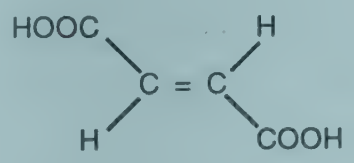

Fig. 4. Structural formula of fumaric acid

Occurrence : Fumaric acid occurs naturally in many plants and is a normal constituent of tissues as an intermediate in the Krebs cycle. It is found in rice, bean sprouts, mushrooms, plant leaves, sugarcane, gelatine and wine.

Preparation : Fumaric acid is manufactured from maleic acid through catalytic isomerisation. Maleic acid is, in turn, prepared from purified maleic anhydride. High purity fumaric acid is obtained through crystallisation of the aqueous mixture, washing and drying. It is also produced by fermentation of molasses or glucose using some *Rhizopus* sp (Butters 1986; Maga and Tu 1995).

Properties : Fumaric acid is a white crystalline, odourless solid. It offers clean tartness and is the strongest of all the

organic food acids. It is sparingly soluble in water but fairly soluble in hot water and alcohol. Quick soluble form (particles of 120 to 320 mesh size) of fumaric acid is also available. It is non-hygroscopic with excellent free-flow properties. It melts at 287°C and has an ionisation constant (25°C) 9.57 $\times 10^{-4}$, pKa value of 3.03, and specific gravity (20°/4°C) of 1.635 (Ash and Ash 1995; Burdock 1997).

Applications : Fumaric acid is used in food industry as a versatile acidifier, curing accelerator and flavouring agent in beverages and other foodstuffs. It has good antioxidant properties as well. Since it is strongly resistant to moisture pick up from the atmosphere, it has excellent free-flow properties, which are exploited by food industry in the preparation of dry mixes. The dry mixes formulated with fumaric acid can be packaged in paper materials to reduce associated caking problems and thus increase shelf stability of the product. Because of its strong acidic character, it is considered a better alternative to citric and malic acids. It is claimed that 2 parts fumaric acid equals 3 parts citric acid in taste and efficiency. Its acidic taste is claimed to be close to the natural tamarind. It blends very well with other food acids without producing a "burst" in taste (Jindra 1989; Burdock 1997).

Fumaric acid is added to a variety of food products including fruit drinks, confections, jams, jellies and citrus marmalade, gelatines and puddings, pie fillings, refrigerated doughs, rye breads, wines, cherries, etc. It has proved to be an excellent flavouring agent for citrus drinks and maraschino cherries. Fumaric acid, although of limited solubility, is considered an acid of preference for gelatine dessert powders, which are generally dissolved in hot water prior to set. For fruit juice drinks, fumaric acid is, however, limited to those applications that do not involve the preparation of stock solutions or beverage concentrates such as single strength non-carbonated beverages. A cold-water soluble fumaric acid is referred to as fumaric acid QD in order to overcome this limitation. This version of fumaric acid provides increased solubility rates. In the manufacture of jam, jellies and other preserves, fumaric acid is added as a slurry because of its limited solubility. The addition is effected after the cook in order to minimise the breakdown of pectin by hydrolysis as well as sucrose inversion. Its antioxidant properties are exploited in arresting the onset and development of rancidity in powdered milk, butter, cheese, sausages, roasted nuts, potato chips, etc (Macrae et al. 1993).

The effective usage levels recommeded for some foods are : non-alcoholic beverages (50 ppm), baked goods (1300 ppm), gelatines and puddings (3600 ppm), jam, jellies, citrus marmalade and other preserves (3000 ppm) (Ash and Ash 1995).

Food regulations and toxicology : Fumaric acid is a GRAS food additive approved by FDA/USDA, Japan and U.K. The ADI as approved by FAO/WHO is 0-6 mg/kg for humans. It is, however, non-toxic to mildly toxic by direct ingestion and skin contact. It is known to be a skin and eye irritant. The LD_{50} (oral) in rats is 10,700 mg/kg body weight (Maga and Tu 1995; Burdock 1997).

Glucono-delta-Lactone

Chemistry : Glucono-delta-Lactone (GDL) also called D-gluconic acid-delta-lactone, is the cyclic 1, 5- intermolecular ester of D-gluconic acid. It has a molecular formula $C_6H_{10}O_6$ and a molecular weight of 178. The structural formula of this lactone is given in Fig. 5 (Maga and Tu 1995).

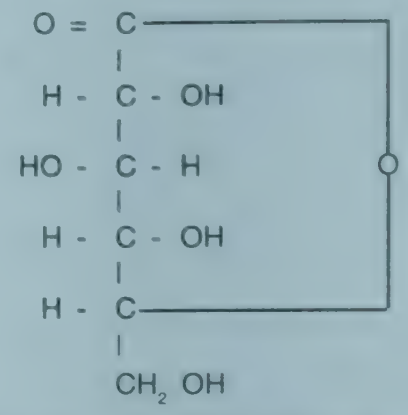

Fig. 5. Structural formula of glucono-delta-lactone

Occurrence : Both GDL and gluconic acid occur widely in natural foodstuffs such as honey, grapes and other fruits.

Preparation : Glucono-delta-lactone is prepared by direct crystallisation from the aqueous solution of gluconic acid. Gluconic acid is produced by the oxidation of D-glucose with bromine water or by the action of suitable enzymes or microorganisms. GDL is produced commercially by a fermentation process using enzymes or micro-organisms like *Aspergillus niger* or *Acetobacter suboxyadans* to convert glucose to gluconic acid (Watine 1995) (Fig. 6).

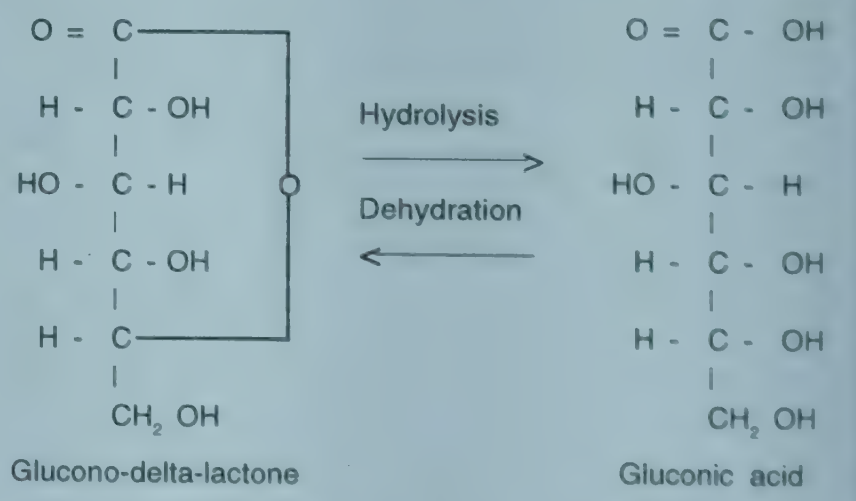

Fig. 6. Conversion process of glucono-delta-lactone to gluconic acid

Properties : The commercial GDL is a white crystalline powder with a neutral taste and is practically odourless. It is soluble in water. In cold water, it hydrolyses slowly as an equilibrium mixture of gluconic acid, its delta and gamma lactones. Its aqueous solution thus possesses acidic taste. Within two hours, the pH of the freshly prepared 1% aqueous solution decreases from about 3.6 to 2.5. It is non-hygroscopic and melts (decomposes) at 153°C. The ionisation constant and pKa values for gluconic acid are 1.99 $\times 10^{-4}$ and 3.7, respectively (Maga and Tu 1995; Burdock 1997).

Applications : GDL is used in food industry for its functional properties of being an acidifier, curing/pickling and leavening agent, for pH control and as flavour enhancer (Maga and Tu 1995; Watine 1995; Burdock 1997). It is a slow-release acidulant with a low acidic flavour and good chelating properties. Because of its slow rate of acidification and mild taste characteristics, it finds special applications different from other acidulants. It is much used in cured meats, frankfurters, salami, sausages, dessert mixes, bakery mixes, processed cheese, cheese whey-based beverages, fish preserves and other fish products, stuffed fresh pastas, processed or pre-cooked meals, fruit and/or vegetable products, salad dressings, sauces, pasta sauces, seasonings and other spice preparations. Its usage level has limitations like any other acidulant e.g. 0.4% in salami and 0.3 to 0.5% in meat.

As the acidulant component of a leavening formulation, it yields gluconic acid at an accelerated rate, as the temperature or concentration is increased during processing and baking operations. Further, the stability of GDL in dry bakery mixes may be increased and the quality of the final baked product is improved by coating the dry lactone with calcium-stearate or vegetable oil.

The stabilisation of the colour of cured meats is attributed to the mild acidity imparted by the gluconic acid released upon slow hydrolysis of GDL. Its use in sausages speeds up the curing process without affecting the emulsion because of its property of gradual lowering of pH. Since it lowers the pH slowly, GDL may have an advantage in the coagulation of milk in the production of cottage cheese, thereby preventing local denaturation and flocculation of proteins and yield a finished product of superior quality. Use of 1% GDL is recommended in yoghurt manufacture to shorten production time, reduce costs and minimise loss of heat unstable components in the milk (Watine 1995).

GDL is found to reduce the bitterness of soybean products. In the manufacture of 'tofu' from soy milk, GDL coagulates the proteins, yielding a fine textured product. Use of 0.4% (w/v) GDL as 16% solution in distilled water is recommended for increased yield of tofu of fine quality. It is proved to be a novel coagulant for the production of yoghurt-like food product from soy milk at a level of 0.1-10% by weight of soymilk.

GDL is also reported to enhance the preservative action of preservatives like benzoic acid, sorbic acid, etc. and their salts. The antimicrobial effect of these preservatives is due to the undissociated molecules. The lower the pH, the higher is the number of undissociated molecules. GDL, owing to its low acidic flavour, permits lower pH values to be reached, resulting in the reduction of the amount of preservative agents needed to achieve the effect.

Enzymatic and oxidative discoloration is a common phenomenon in seafoods and vegetables. GDL, as an acidulant can help to prevent this discoloration in combination with other products. In canned crab, GDL can be used to replace EDTA and phosphate. In the preservation of shrimps, application of sulphite may be reduced to the extent of 50% by an equivalent quantity of GDL. Similarly, the use of GDL can substantially reduce the use of sulphiting agents in other products. In a patented process, the American National Can is reported to have replaced 0.5% sulphite by a combination of 1.5% GDL and 0.05% sulphite (Watine 1995).

The use of GDL in combination with acids is advocated to lessen the drastic commercial sterilisation of low-acid foodstuffs so that flavour, colour and texture of the foodstuff more closely resemble those of the fresh home-cooked product. In salad dressings, it is recommended to partially replace vinegar for effective preservation by adjusting the pH to less than 4 and impart, at the same time, a flavour profile for wider consumer acceptance. Thus, it can be added to any food product that requires acidification but a mild flavour profile. Some other examples are canned products, pancakes batter, sauces and mayonnaise.

Food regulations and toxicology : Glucono-delta-lactone has been accorded GRAS status as food ingredient. It is approved by FDA/USDA, U.K., Japan and is listed with EEC. The European proposal for a directive on food additives other than sweeteners and colours, includes GDL in Annexure 1 "Generally Permitted Food Additives". This means that GDL is approved for use in all kinds of foodstuffs except for unprocessed foods and infant foods. However, the maximum level of GDL allowed in the final product is 3000 mg/kg. The ADI is 0-50 mg/kg body weight calculated as gluconic acid. Since GDL ionizes to form gluconic acid, an oxidation product of glucose, which is the normal dietary carbohydrate and the principal energy source in human nutrition, it is not expected to pose any serious health hazards to humans. However, it may cause eye irritation upon direct contact. The LD_{50} (intravenous) in rabbit is reported to be 7630 mg/kg body weight (Maga and Tu 1995; Watine 1995).

Lactic Acid

Lactic acid (2-hydroxypropanoic acid) also known as milk acid, is a monocarboxylic organic acid with a molecular formula $C_3H_6O_3$ and a molecular weight of 90.08. It is optically active and structural formulae of different isomers are given in Fig. 7 (Kirk-Othmer 1995; Maga and Tu 1995).

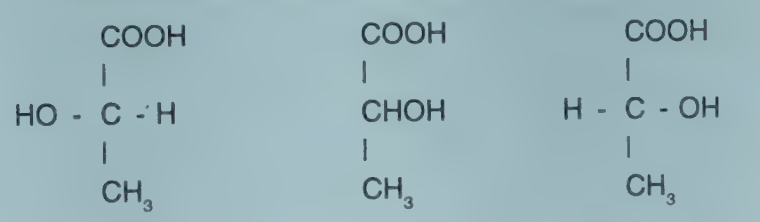

Fig. 7. L - (+) Lactic acid DL - Lactic acid D - (-) Lactic acid

Occurrence : Lactic acid is widely found in many foods both naturally or as a product of *in situ* microbial fermentation e.g. yoghurt, buttermilk, cheese, pickles, sauerkraut, sourdough breads, beer and many other fermented foods. It is a principal metabolite in most living organisms, from anaerobic prokaryotes to humans. It occurs in the juice of muscular tissue, bile, etc. It is also a major component of corn steep liquor, a by-product of the corn-wet-milling industry (Butters 1986; Kuipers 1992).

Preparation : Lactic acid can be manufactured either by carbohydrate fermentation or by chemical synthesis using

lactonitrile route. A wide variety of carbohydrate sources e.g. molasses, corn syrup, whey, dextrose, sucrose etc. can be used for commercial production through fermentation process using homolectic organisms like *Lactobacillus delbrueckii, L. bulga-ricus,* and *L. leichmanii* (Kuipers 1992; Maga and Tu 1995).

It is generally marketed under four product categories namely, synthetic - a highly purified product, foodgrade product derived from carbohydrate fermentation, technical - a crude product obtained from either of the processes. The foodgrade lactic acid is marketed as 50, 80 and 90% aqueous solutions and in a powdered from (Butters 1986; Kuipers 1992).

Properties : Lactic acid is optically active having three isomers namely, L - (+), D- (-), and DL-forms. The pure, anhydrous lactic acid is a white crystalline solid. It is highly miscible with water and alcohol. Its aqueous solution is colourless or yellowish, hygroscopic syrupy liquid. It is non-volatile and practically odourless with an acrid taste. The crystalline lactic acid melts at 16.8°C and its aqueous solution boils at 122°C (15 mm). It has a specific gravity (20°C) of 1.2243 g/ml, viscosity 36.9 mPa.s, ionisation constant (25°C) 1.374 10^{-4} and pKa value of 3.86 (Maga and Tu 1995; Burdock 1997).

Applications : Lactic acid is used as acidifier, pH control agent, curing and pickling agent, flavour enhancer and flavouring agent and inhibitor of microbes (Dziezak 1990; Kuipers 1992; Maga and Tu 1995; Burdock 1997). The favourable properties of lactic acid that make it suitable for food applications are :

(a) a mild acid taste in contrast to sharp taste of most other food acids, (b) does not mask or overpower the weaker aromatic flavours of foods, (c) has a distinct preservative action and regulates the microflora and (d) occurs naturally in many foodstuffs, thus introducing no foreign element to the food.

Therefore, it is the acidulant of choice in many food products viz., dairy products including immitation dairy products such as cheese, margarine, yoghurt powder, meat products, bakery products, confectionery products, beverages and sherbets, jam and jellies, pickles, soups, mayonnaise, processed eggs, and a host of other processed foods. It is the preferred acidulant for adjusting acidity and ensuring the clarity of brines for pickles and olives. To packaged Spanish olives, it is added to inhibit spoilage and further fermentation during storage. Partial replacement of acetic acid with lactic acid in pickled vegetables improves microbial stability.

Sugar confectionery : The use of lactic acid in the continuous production of high-boiled sweets is preferred. The buffered acid can be introduced easily into the molten sugar syrups even at high temperatures used in depositing lines without the risk of increased sugar inversion. The resulting sweets are perfectly clear and no air is trapped. Sugar inversion causes stickiness and cool flow. In wine gums, lactic acid is of choice owing to its flavour enhancing effect (Dziezak 1990; Kuipers 1992; Maga and Tu 1995).

Bakery products : In the manufacture of hard-type biscuits and cream crackers, cream of tartar generally used may be replaced with lactic acid. Use of 0.45 and 0.30% (wheat flour basis) lactic acid in hard-type biscuits and cream crackers, respectively gives higher specific volume and a reduced sponge fermentation time compared to cream of tartar. It can be used for direct acidification of rye or rye-wheat breads (Dziezak 1990; Kuipers 1992; Maga and Tu 1995).

Beverages : In delicately flavoured soft drinks and fruit juices, lactic acid is used in place of citric acid because of its milder taste. Lactic acid is preferred in the acidification of low acid wines and ciders, as it is not attacked by acid-degrading bacteria. In the manufacture of beer, lactic acid is preferentially added to adjust the pH during the mashing process and during wort cooking (Dziezak 1990; Kuipers 1992; Maga and Tu 1995).

Food regulations and toxicology : The use of lactic acid in foods is limited by good manufacturing practice. The food-grade lactic acid should meet the Food Chemicals Codex III specifications. It can be determined by titration with sodium hydroxide and by HPLC. The material should contain not less than 95% and not more than 105% of the labelled concentration of lactic acid. It should not contain more than 0.1% sulphated ash, 0.2% chloride, 0.25% sulphate, 10 mg/kg iron, 3 mg/kg arsenic and 10 mg/kg heavy metals. It is listed as GRAS by FDA/USDA, Japan, U.K. and EEC. The Fruit Products Order (1995) permits it to be added in fruit and vegetable products as per good manufacturing practice. There is no limit for acceptable daily intake by humans. However, it is not recommended for use in infant foods. It is moderately toxic by direct ingestion and is a severe skin and eye irritant. The LD_{50} (oral) for rats is 3730 mg/kg body weight (Maga and Tu 1995; Burdock 1997).

Malic Acid

Chemistry : Malic acid (1-hydroxy-1, 2-ethanedicarboxylic acid) or hydroxysuccinic acid is also known as pomalous or apple acid. It is optically active having three isomeric forms. It has molecular formula $C_4H_6O_5$ and its molecular weight is 134.09. The three isomers are represented by the structural formulae (Fig. 8) (Kirk-Othmer 1995; Maga and Tu 1995).

Occurrence : Malic acid is a natural constituent and

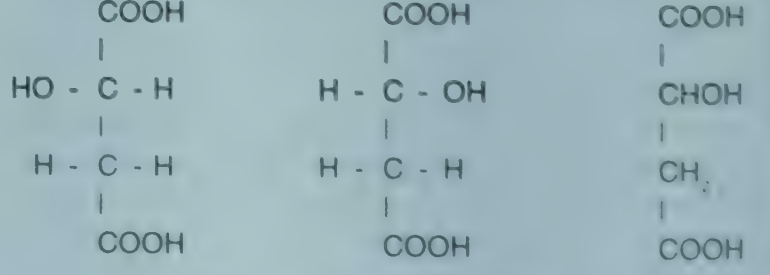

Fig. 8. L - (-) Malic acid D - (+) - Malic acid DL -Malic acid

common metabolite of plants and animals. Among the fruits, watermelon, plum, apple, cherry, peach, banana, pear, grape, apricot, gooseberry, and pineapple, respectively contain 100, 98.5, 97.2, 94.2, 50-96, 54-92, 33-87, 60, 24-70, 46, and 12.5 % of the total acids present in the individual fruit. It is the second largest acid in citrus fruits, figs, berries, beans and tomatoes. Potatoes, carrots, peas, broccoli and rhubarb too

contain this acid (Dziezak 1990; Ash and Ash 1995; Maga and TU 1995; Burdock 1997).

Preparation : It is commercially produced by the catalytic hydration of maleic and fumaric acids. Malic acid is then recovered from the equilibrium product mixture (Butters 1986; Maga and Tu 1995). Compared to the naturally occurring malic acid which is levorotatory, the synthesised product is a racemic mixture of D-and L-isomers. The racemic form of the acid is widely used in food industry, primarily in foods (27%) and beverages (55%).

Properties : Malic acid is a white crystalline powder, very soluble in water and alcohol. It has a clean tart taste with no flavour of its own. It is non-hygroscopic under ordinary conditions but hygroscopic under high humidity conditions. It melts at 100°C (d-orl-form), 128°C (dl-form), and boils at 140°C (d-orl-form), 150°C (dl-form). It is a relatively strong acid with ionisation constant (25°C) of 4×10^{-4} and a pKa value of 3.40 (Dziezak 1990; Maga and Tu 1995).

Applications : Malic acid is widely used as an acidulant, flavour enhancer and flavouring agent and for pH control (Butters 1986; Dziezak 1990; Maga and Tu 1995; Burdock 1997). Because of its unique combination of properties such as taste-blending characteristics, flavour-fixing qualities, ability to retain sour taste longer, high water-solubility, and buffering properties, it is used in a variety of foods and beverages. It is the modern replacement of citric acid. Although, its ionisation potential is similar to that of citric acid, malic acid has a stronger apparent acidity, which qualifies smaller amounts (80-90% by weight of citric acid) of it to be used in certain applications for the same taste effect (Table 3).

Beverages : Malic acid is being increasingly used in both liquid and powder beverage mixes as acidifier, flavour enhancer, and pH buffer. It has stronger acid taste than citric acid. It suppresses the bitter after-taste in low calorie drink formulations that contain saccharine as sweetener. It is known to provide a stronger, yet slower and longer flavour release in comparison to citric acid. It promotes harmonious blending of combination flavours such as apple/raspberry, and orange/pineapple eliminating individual flavour sensation.

Other foods : It is also used in jams, jellies and preserves to balance flavour and adjust the pH for pectin set. It is used in candy, chewing gum, fats and shortenings, fruits, gelatines, puddings, sherbets and water ices and wine. In candy manufacture, malic acid is used because of its low melting point and high solubility and to impart a tang or tartness compatible with most hard candy flavours. In wine and cider, it is used to impart bouquet and adjust the pH.

The recommended level of malic acid to be used in different food products varies with the nature of the product, for example, beverages (3.4%), chewing gum (3%), soft candy (3%), hard candy (6.9%), gelatines and puddings (0.8%), jams (2.6%), processed fruits and fruit juices (3-5%), other foods (0.7%). Its use is limited by good manufacturing practice in canned apple sauce, other canned fruits and vegetables, fruit juices and concentrates, quick-frozen fruits and vegetables (Ash and Ash 1995; Maga and Tu 1995).

Food regulations and toxicology : Malic acid is a GRAS substance for its use as an acidulant, flavour enhancer, flavouring and pH control agent. Since it has well-established metabolic pathway, FAO/WHO has not set any limit for the acceptable daily intake of the L-(-)-isomer of malic acid, while D-(-)-isomer is conditionally allowed at 0-100 mg/kg body weight/day in the human diet. It, however, may not be used in baby foods. It should meet the specifications of Food Chemicals Codex. In India, it is approved under Prevention of Food Adulteration Act (1954) and Fruit Products Order (1995) for use in soft drinks, confectionery, jams and jellies, dry beverage mixes, fruit juices, etc. The Fruit Products Order permits it to be added to fruit products as per good manufacturing practice (Dziezak 1990; Maga and Tu 1995).

It is reported to be moderately toxic by ingestion and is a severe irritant to the skin and the eyes. LD_{50} (oral) in rat is 3730 mg/kg body weight.

Phosphoric Acid

Chemistry : Phosphoric acid (orthophosphoric acid) is a tribasic inorganic acid with a molecular formula H_3PO_4 and a molecular weight of 98.00. Its structural formula is given in Fig. 9 (Kirk-Othmer 1995).

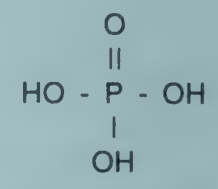

Fig. 9. Structural formula of phosphoric acid

Occurrence : Phosphoric acid is one of the components of the energy transfer systems in the living cell. The phosphate ion plays a vital role in the synthesis of compounds essential to life. It is found in various types of nucleic acids vital to genetic reproduction and to restoration of injured tissues. It is an active component of many enzyme systems. Phosphate ion is also involved in the synthesis and the metabolism of carbohydrates, fats, proteins, and in the formation and structure of such tissues as brain, muscle, and skeleton. However, no living organism is capable to synthesize the phosphate ion. It must necessarily be absorbed through the food supply. Plants derive it from the soil and the animals/humans in turn get it from the plants (Dziezak 1990; Maga and Tu 1995; Burdock 1997).

Preparation : Phosphoric acid is manufactured commercially from phosphate rock derived from the fossil bones of animals. The phosphate is treated with sulphuric acid to release the acid, which is then concentrated and purified. It is generally marketed as a liquid of varying concentrations (Butters 1986).

Properties : Pure (100%) phosphoric acid is a white crystalline powder that melts at 42.35°C. As liquid, it has clear, syrupy appearance. It is odourless but possesses acrid taste. It is non-hygroscopic in nature. It is strongest of all food acids giving the lowest attainable pH. It is soluble in water and alcohol. In dilute solution, phosphoric acid has pleasingly sour

taste resembling the taste of acetic and citric acids but without any fruity flavours. An aqueous solution (100%) of phosphoric acid has boiling point at 261°C, specific gravity (25°C) 1.864, and viscosity 28 mPa.s. The acid has ionisation constants (25°C) K1 = 7.52×10^{-3}, K2 = 6.23×10^{-8} and corresponding pKa values of 2.12 and 7.21. The technical grade phosphoric acid contains 57% P_2O_5. The foodgrade phosphoric acid is marketed as 75, 80 and 85 % aqueous solutions (Dziezak 1990; Maga and Tu 1995; Burdock 1997).

Applications : Phosphoric acid is used in food industry for its functional properties as an acidulant, flavouring agent and flavour enhancer, pH control and buffering agent. It is used widely in cola, root beer, sarsaparilla and other similarly flavoured carbonated beverages as a tart flavouring agent. This harsh, biting-tasting acid compliments the cola flavour especially. The low pH imparted by this acid further accentuates the cola flavour. The basic properties of the acid provide minor buffering capacity as well in the beverage. It is also used as a general protein acidulant, in cheese production as coagulant, acid flavouring and buffering agent in jams and jellies, in bread doughs and cake flours, confectionery products, in brewing to adjust pH, to neutralize caustic in the peeling of fruits and in processing of oils and fats to control pH and bind metal ions. In the manufacture of jams and jellies, the use of phosphoric acid as a buffering agent ensures the strongest gel strength and checks the dullness of the jelly colour by chelating the metal ions (Maga and Tu 1995; Burdock 1997).

Food regulations and toxicology : Phosphoric acid has been accorded GRAS status as food ingredient. It is approved by FDA/USDA, U.K., Japan and listed by EEC for use in foods and beverages. The acceptable daily intake is 0-70 mg/kg body weight/day for humans. It is non-toxic to moderately toxic by direct ingestion and skin contact. It is a skin and eye irritant. LD_{50} (oral) in rats is 1530 mg/kg body weight (Maga and Tu 1995).

Tartaric Acid

Chemistry : Tartaric acid, L-2, 3-dihydroxybutanedioic acid or 2, 3-dihydroxysuccinic acid, is a dihydroxydicarboxylic acid with two chiral centres. It is optically active and exists as dextro-and levorotatory acid, the mesoform (inactive), and the racemic mixtures. Tartaric acid that occurs in grapes and tamarind fruits is the natural dextrorotatory form, L-(+)-tartaric acid. It has a molecular formula $C_4H_6O_6$ and a molecular weight of 150.09. The structural formulae of the three optically active forms are given in Fig. 10 (Kirk-Othmer 1995; Maga and Tu 1995).

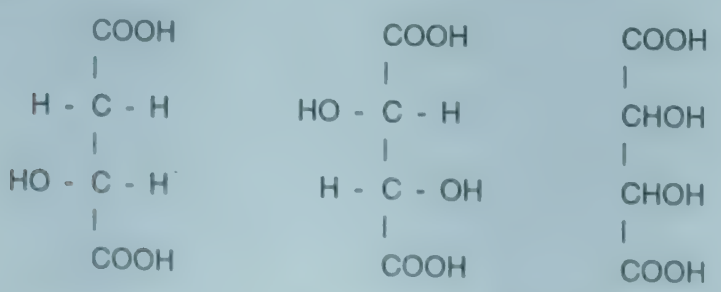

Fig.10. L - (+) Tartaric acid | D - (-) - Tartaric acid | DL - Tartaric acid

Occurrence : It occurs either in free state or as potassium or calcium tartarate in many fruits and vegetables such as grapes, tamarind, pineapple, mulberries, gherkins. Grapes and tamarind are the rich source of this acid. The edible portion of the ripe tamarind fruit contains 8-10 % total tartaric acid. The argols- a by-product of grape juice/wine manufacture industry, contain 45-70 % tartaric acid (Macrae et al. 1993; Maga and Tu 1995; Burdock 1997).

Preparation : Tartaric acid is manufactured from argols, press cakes and lees-by-products of grape-juice/wine manufacturing industry. These by-products are treated with hydrochloric acid, and the solution is then nearly neutralized with milk of lime, $Ca(OH)_2$ which precipitates calcium tartarate leaving behind potassium tartarate in solution. Calcium tartarate is subjected to purification, followed by treatment with dilute sulphuric acid, which precipitates calcium sulphate leaving tartaric acid in solution. The calcium sulphate is filtered off, and the tartaric acid solution is concentrated to crystallisation (Maga and Tu 1995; Burdock 1997).

Properties : Tartaric acid is a colourless, odourless crystalline powder with a strong, tart acidic taste. The common D-tartaric acid found in nature is optically active and crystallizes in large prisms as a monohydrate. It is non-hygroscopic. It is highly soluble in water (139 g/100 ml water at 20°C), and alcohol. It has a melting point at 168-170°C (anhydrous), specific gravity (20°/4°C) 1.7598, ionisation constants (25°C) K1 = 1.04×10^{-3}, K2 = 4.55×10^{-5} with corresponding pKa values of 2.98 and 4.34 (Maga and Tu 1995; Burdock 1997).

Applications : Tartaric acid is a direct food additive used as an acidulant, pH control agent, flavouring agent and flavour enhancer. It is generally used where high tartness is required, for example, non-alcoholic beverages, candies, gelatine desserts, jams and jellies. Its use in grape and lime-flavoured beverages is preferred because it potentiates the flavour of the beverage. Confectionery manufacturers too prefer to use tartaric acid in grape-flavoured candy for high relative tartness desired to potentiate grape flavour. In the manufacture of jams, jellies and other preserves, tartaric acid is added as 50% solution after the cook in order to minimise sucrose inversion and hydrolytic breakdown of pectin. In starch jellies, it tends to modify the flow properties of the candy mass, when used in conjunction with "cream of tartar". It is also used in baking powder, fruit *purees,* stewed fruits, etc (Ash and Ash 1995; Burdock 1997).

The recommended usage level of this acid varies with the food product such as non-alcoholic beverages (960 ppm), candy (5400 ppm), baked goods (1300 ppm), ice-cream (5700 ppm), gelatine and puddings (60 ppm), chewing gum (3700 ppm), condiments (10,000 ppm) (Ash and Ash 1995).

However, because of high prices and short supplies, the use of tartaric acid in food industry has been decreasing.

Food regulations and toxicology : Tartaric acid is a GRAS food additive but it should meet the specifications of Food Chemicals Codex. The material after drying over P_2O_5 for 3 h should contain not less than 99.5% of L - (+) - tartaric acid, specific rotation $(\alpha)^{20}$ D = + 11.5°C to 13.5°C. It can be

assayed by titration against I N NaOH. It should be free of oxalate and should not contain more than 0.1% sulphated ash, 0.05% sulphate, 3 mg/kg arsenic, and 10 mg/kg heavy metals. The Fruit Products Order (1995) permits it to be added to fruit products as per good manufacturing practice. The acceptable daily intake as set by FAO varies from 0-30 mg/kg body weight for L- (+) - tartaric acid in human dietary. It is reported to be mildly toxic by ingestion as such. However, it is poorly absorbed from the intestine and is excreted unchanged in urine. LD_{50} (intravenous) in mouse is 485 mg/kg body weight (Ash and Ash 1995; Maga and Tu 1995).

Criteria for Selection

Food industry has a range of acidulants to choose from. An apt choice will ensure optimum quality finished food product. Following are some of the aspects that may be considered as guide, while selecting an acidulant for a particular food application (Jindra 1989; Dziezak 1990; Macrae et al. 1993; Burdock 1997).

Physical form : The physical form, whether liquid or solid, in which an acidulant is available to the food industry, limits its use for some applications. For example, liquid acidulants though easy to handle, pump and measure within the manufacturing area, these cannot be used for solid food mixes and formulations. Therefore, the use of adipic and fumaric acids is recommended in formulating baking powders, and dry bakery mixes. Similarly, for dry beverage mixes, citric, malic and fumaric acids are preferentially used. Fumaric acid, which has limited solubility in water, may be presented in the form of ultrasphers, which have improved solubility. Further, innovations like co-granulated citric, fumaric, and malic acids allow for variation in tartness in the finished product. Similarly, encapsulated food acids may be preferred for their use in candy and chewing gum in which slow release of the acid provides prolonged sourness, flavour and juiciness, while being chewed or masticated.

Solubility : Solubility and the rate of solubility of an acidulant are both important issues to consider, when selecting an acid for a specific application. The acidulant of choice should be soluble at the concentrations needed at stipulated temperatures and within a time frame. The absolute solubility of citric acid and malic acid, for example, allows the convenient preparation of 50 % stock solutions to facilitate their addition to beverage concentrates. Further, high solubility rates make citric and malic acids ideal candidates for use in dry beverage mixes where rapid dissolution is desired. Fumaric acid, on the other hand, is misfit for such applications. A cold water soluble version of fumaric acid has, however, been developed with a higher price tag.

Hygroscopicity : An acidulant having hygroscopic properties will be a misfit for use in dry mix food products. It will be of particular concern to many beverage manufacturers. Fumaric acid, due to its non-hygroscopic nature, can serve both as an acidulant and as a free-flowing agent in dry mix products e.g. baking powders, leavening systems, cake mixes, beverage mixes etc. Dry mixes formulated with fumaric acid can be packaged in paper materials to reduce associated caking problems, thereby increasing the shelf-stability of the product.

Functionality : There are subtle differences in the nature of tartness and acid strength of the available acidulants (Tables 2 and 3). The acidulants of choice should be strong enough to display required functionality e.g. tartness, control of pH and acidity. Citric acid is used where "burst" of tartness is desirable. Malic acid, on the other hand, has a stronger apparent acidity without imparting "burst" in flavour, which permits smaller amounts of it to be used for certain applications e.g. hard candy manufacture. Lactic acid, because of its mild acid taste, is preferentially employed in the manufacture of delicately flavoured soft drinks and fruit juices. For the same reason, lactic acid eliminates the risk of sucrose inversion in candy manufacture, yielding perfectly clear candies. In wine gums, lactic acid is preferred owing to its flavour enhancing effect. Further, its use is preferred in the acidification of low acid wines and ciders, as it is not attacked by acid-degrading bacteria as are tartaric, malic and citric acids. The use of glucono-delta-lactone is recommended where gradual decrease in pH is desirable e.g. manufacture of cottage cheese, yoghurt and tofu from soy-milk.

Specificity or compatibility : The acidulant of choice should possess clean taste, free of off-notes that are foreign to foods. In other words, it should not affect the overall flavour profile of the finished product. Phosphoric acid is the acidulant of choice in the manufacture of carbonated cola drinks and root-beer because of its sharp, tingling tart taste. Citric and malic acids are recommended for their use in fruit-flavoured carbonated beverages because they complement fruit flavours. Tartaric acid, on the other hand, is preferentially employed in grape-and lime-flavoured beverages for flavour enhancement. In the manufacture of non-carbonated beverages e.g. fruit drinks, fruit nectars, etc., the acidulants are selected based on flavour requirements and compatibility. Citric, fumaric, tartaric and malic acids are commonly used in such types of beverages. In the manufacture of low-calorie beverages, citric and malic acids can be used to control pH of the beverage so that desired sweetness characteristics are attained. Adipic and fumaric acids are preferred for their use in gelatine dessert mixes because they promote optimum gel strength and protect gelatine desserts against loss of quality.

Environmentally friendly : The acidulant used during manufacture of food products should not, in any way foul the environment in the factory causing discomfort to the workers. It should be non-toxic. Also, it should not aggravate pollution and waste disposal problems.

Cost and availability : The acidulant used in any food application should be cost effective so that the final price of the finished product does not need to be unreasonably escalated. The product should withstand the market competition. Sustained availability of the acidulant is equally important for production to go on without interruption.

Conformity to food regulations : Like all other food additives, the applications of acidulants in food industry are also governed by food acts and regulations. It is, therefore,

mandatory for food industry to use only those acidulants that are officially approved. The acidulant should preferably be 'generally recognised safe' (GRAS) for its intended application. GRAS acidulants may be used in foods not covered by standards of identity and have no imposed restrictions on their usage level, provided that good manufacturing practices are observed. There are other acidulants like fumaric acid, regulated as a food additive but are not GRAS. The usage of such substances is restricted to levels reasonably required to achieve the intended effect. The food industry should also be aware of the fact that the foods meant for a specified group of consumers e.g. babies or infants, have imposed restrictions on the use of certain acidulants. It should also be known, if there are any local or state regulations that may restrict the selection of an acidulant for addition to a specific food product.

Also, the food processor must maintain a good liaison with the manufacturers and suppliers of the acidulants. They are the sources of detailed information on the physico-chemical properties, approximate usage level and methods of incorporating the acidulant into the food product, the knowledge of which ensures optimum quality of the finished product.

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RESEARCH PAPER

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Effect of Blanching on Pickled Bitter Gourd (*Momordica charantia*)

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Bitter gourd (*Momordica charantia* Linn.) fruit slices blanched either in 5% NaCl at 100°C for 3 min (HTST) or in a two-stage balnching process in 5% NaCl at 70-80°C for 20 min (LTLT), followed at 100°C for 3 min (HTST) was most effective in removing bitterness and maintaining firmness of vinegar pickled products. Effect of blanching was significant ($p<0.05$) on the acceptability of the products. A product from two-stage blanching treatment with 0.1% $CaCl_2$ got the highest scores (7.83-8.17) and was rated the best. On storage at 30-32°C for 32 days, pH stabilised at 3.17-3.20, ascorbic acid decreased, colour changed from dark green to olive green with no microbial growth.

Keywords : Bitter gourd, Blanching, Vinegar, Pickle, Firmness, Acceptability.

Bitter gourd (*Momordica charantia* Linn.), a member of the *Cucurbitaceae,* family is also known as bitter cucumber, bitter lemon, caraille, alligator pear, maiden apple coucouli, balsam pear or karela (Tindall 1983; Yamaguchi 1983). Best known for its medicinal properties (Ayensu 1981), the fruit though bitter is valued as a vegetable when young (Kalra et al. 1988). While most West Indian consumers find this bitterness to be undesirable, they consume the fruit as a vegetable either fried, curried, baked or stuffed with meat. The seeds of the ripe fruit are used as condiment (Kalra et al. 1988). The fruits are harvested while still immature, usually white to green in colour and 50-70 days from sowing (Tindall 1983). As the fruit matures beyond its marketable stage, undesirable changes occur such as loss of skin lustre, changes, in skin colour and hardening of the seed coat. The fruit has a high moisture content, large surface area to volume ratio and a thin cuticle, thus making it susceptible to physical injury and moisture loss. Under tropical ambient conditions, rapid senescence, is seen as early signs of yellowing. This is followed by ripening, which is characterised by fruit softening, bright yellow colour and the development of red pigmentation in the arils (Mohammed and Wickham 1993).

Processing of bitter gourd into a pickled product is very important to upgrade its commercial value by increased utilisation, extending its availability, decreasing the undesirable bitterness and adding variety and value to the raw product. According to Kalra et al (1988), very limited work has been done on the processing of bitter gourd. Hence, this study was undertaken to investigate the effects of blanching treatments on the bitterness and texture of pickled bitter gourd and to evaluate the quality changes during storage of an organoleptically acceptable fresh pack pickle.

Materials and Methods

Bitter gourd : Freshly harvested immature bitter gourd (*Momordica charantia* Linn.) were sorted for freedom from blemishes, firmness, uniformity of green colour and size (20-30 cm length × 10-20 cm diameter). Fruits were washed in sodium hypochlorite solution (500 ppm), air dried, sealed in low density polyethylene bags (LDPE), packaged five fruits per bag, stored at 11 ± 1°C and processed within 24 h of harvesting.

Processing : The processing scheme used for pickling bitter gourd is shown in Fig. 1. The various pre-treatments applied to the bitter gourd rings are given in Table 1. Stems, seeds and endocarp were removed and the fruits cut crosswise (approximately 7 mm width and 1 cm thickness). To bitter gourd rings (200 g) filled into sterilised glass bottles (500 ml), hot pickling solution was poured, leaving a headspace of 1 cm. The various formulations of pickling solution are shown in Table 2. The combined ingredient was heated to 90°C for 2 min prior to pouring into bottles. The pickled products were

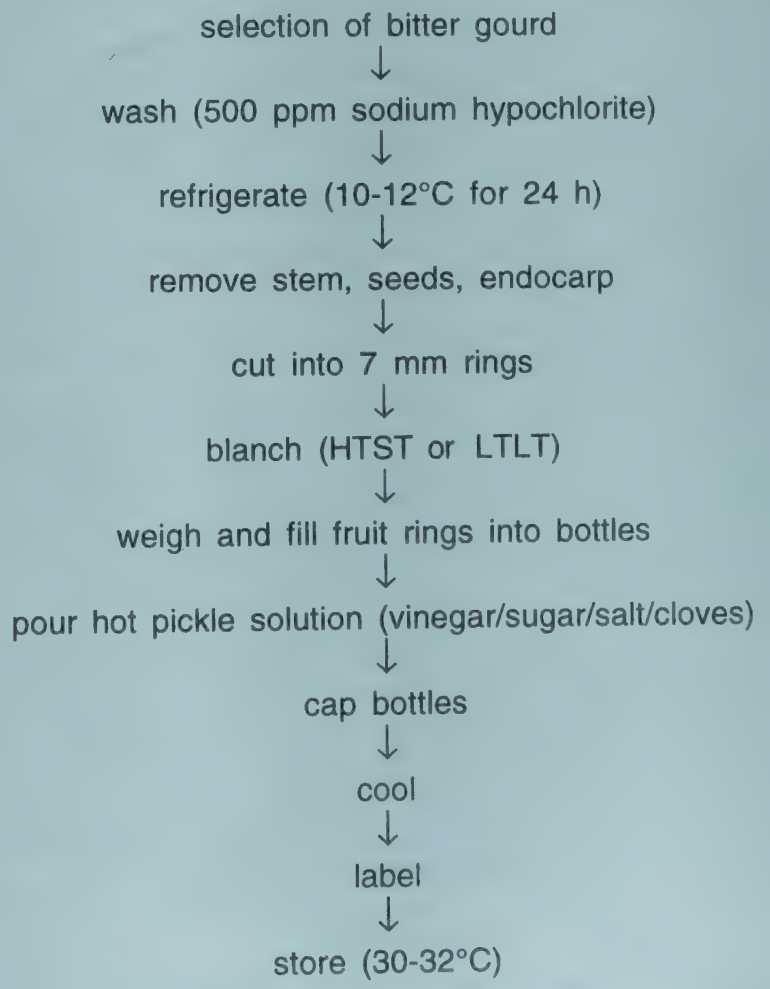

Fig. 1. Processing steps for pickled bitter gourd

* *Corresponding Author*

TABLE 1. PRE-TREATMENT PROCESSING APPLICATIONS TO BITTER GOURD

Treatment No.	Soaking in NaCl	Blanching method	Comments on UA** or A*
1	no soaking	no blanching	extremely bitter, raw taste, too crunchy (UA)
2	no soaking	blanch in water at 100°C for either 3 min or 5 min	3 min-bitter, good texture (UA); 5 min-too soft (UA)
3	no soaking	blanch in 5% NaCl solution for either 3 min or 5 min	3 min-bitter, good texture (A); 5 min-soft texture (UA)
4	no soaking	blanch in 10% NaCl solution at 100°C for either 3 min or 5 min	bitter, firm texture (UA)
5	no soaking	blanch in water at 70-80°C for 20 min and at 100°C for 3 min	bitter, firm texture (UA)
6	no soaking	blanch in 5% NaCl at 70-80°C for 20 min and then at 100°C for 3 min	not bitter, firm texture (UA)
7	no soaking	blanch in 10% NaCl 70-80°C for 20 min and then at 100°C for 3 min	not bitter, firm texture (UA)
8	soak in 5% solution for 24 h at 30-32°C	blanch in water at 100°C for either 3 min or 5 min	not bitter, rubbery texture (UA)
9	soak in 10% NaCl solution for 24 h at 30-32°C	blanch in water at 100°C for either 3 min or 5 min	not bitter, salty, soft texture (UA)

**UA - unacceptable; A - acceptable

Treatments # 3 and #6 were selected for further experimental work

TABLE 2. BITTER GOURD PICKLING SOLUTION FORMULATIONS

Formulation	Pickling	Comments	acceptable/ unacceptable
# 1	1L vinegar, 250 g sugar, 5 g salt, 3 g cloves	not sweet; more salt required sourness of vinegar was acceptable	unacceptable
# 2	1L vinegar, 500 g sugar, 5 g salt, 3 g cloves	bitter; acceptable sugar; more salt required; sourness of vinegar acceptable	unacceptable
# 3	1L vinegar, 500 g sugar, 5 g salt, 3 g cloves	acceptable sugar; more salt required; sourness of vinegar acceptable	acceptable

Formulation # 3 was chosen for further experimental work

judged by panelists for maintenance of firmness and removal of bitterness. According to Lin and Schyvens (1995), texture is one of the most important attributes affecting the acceptability of processed vegetables and fruits.

The two pre-treatments that were selected for further experimental work in pickling of bitter gourd required no prior soaking in NaCl. Table 1 indicates that these treatments were a high temperature and short time (HTST) treatment, blanched in 5% NaCl solution at 100°C for 5 min (Treatment 3) and a two stage blancing process (Treatment 6) blanched in 5% Nacl solution at 70-80°C for 20 min (low temperature and long time, LTLT) followed by 100°C for 3 min (high temperature and short time, HTST). Pickling formulation (#3) selected consisted of 1L acetic acid (5% vinegar), 500 g sucrose, 10 g NaCl and 3 g clove without or with addition of 0.1% $CaCl_2$ $2H_2O$ w/v. According to Kalra (1990), the reasons for blanching before processing were to inactivate enzymes, remove raw or bitter flavour, stabilise the colour and reduce bacterial load. Also, Breslin and Beauchamp (1995, 1997) reported on the role of salts in potentiating other flavours by suppressing bitterness or enhancing sweetness.

Physico-chemical analyses : All physico-chemical analyses of the product were measured on days 0, 12, 22 and 32 after processing and on storage at 30-32°C.

Texture was measured according to the operating instructions of the Koehler Instrument Company (1991) on a Koehler digital penetrometer (model K19550, Koehler Instrument Company, Inc., Bohemia, NY, USA) using a 47.5 g plunger with an attached penetration needle (model K 20500). Penetration time was set at 0.5 sec and an average of four measurements was recorded.

Colour was measured on a Minolta Chroma Meter (CR-200B, Minolta Camera Corp., Ramsey, NJ, USA). The chromaticities of the fruits and rings were measured as 'L', 'a', 'b' coordinates where 'L' represented lightness or darkness (the higher the value, the lighter the colour), + 'a' = redness, - 'a' = greenness, + 'b' = yellowness and - 'b' = blueness for colour (Francis 1998). The a:b ratios represented minor variations or shifts in colour (Table 3).

The pH of the filtrate was measured in triplicate on an Orion pH meter (model EA 920, Orion Research Inc., Boston US) using 10 g sample of the product blended in 10 ml distilled water. The meter was standardised using pH 4.0 buffer and a pH 7.0 buffer (Sadler and Murphy 1998).

Ascorbic acid content (mg/100g fresh weight) was determined by the iodate titrimetric method (Food Chemical Codex 1966).

Microbial analyses : Microbial analyses were done by serial dilutions of the pour plate method using Plate Count Agar (PCA) for total counts of microorganisms and Potato Dextrose Agar (PDA) for yeasts and moulds as outlined by Benson (1990). All media were obtained from Oxoid Unipath Ltd (Hampshire, UK). The PCA plates were incubated at 28°C for 48 h and PDA plates at 32°C for 48 h. Selected plates containing 25-250 colonies were counted after the incubation

period and expressed as cfu/g (Swanson et al. 1992).

Sensory evaluation : Sensory evaluation was conducted to screen the acceptable pickled products based on removal of bitterness and the maintenance of a crunchy texture. The two most acceptable products, treatment 3 (T3; no prior soaking in NaCl and blanching in 5% NaCl solution at 100°C for 3 min, Table 1) and treatment 6 (T6; no prior soaking in NaCl and blanching in 5% NaCl solution at 70-80°C for 20 min, then blanching at 100°C for 3 min, Table 1) without the addition of 0.1% $CaCl_2$ or with the addition of 0.1% $CaCl_2$ (T3C; T6C) were randomly coded and rated by panelists on a 9-point Hedonic scale (9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely) according to the method of Watts et al (1989). Samples were served with crackers and water for cleansing the palette between samples. The product which was rated with the highest Hedonic scores for acceptability on storage was then subjected to a paired preference test with a commercial pickled product. Panelists were asked to select the sample most preferred as described by Resurreccion (1998) and to give any descriptive comments on the experimental product.

All tests were conducted in individual partitioned booths with samples served through windows from the preparation room in the Sensory Evaluation Laboratory of the Department of Food Production, The University of the West Indies, Trinidad under fluorescent lighting. The twelve semi-trained panelists were selected from the staff and students of the University of the West Indies, based on familiarity to sensory evaluation and consumption of pickled products.

The experiment was replicated and the data were analysed by two-way analysis of variance (ANOVA) and the differences among the means of treatments separated by Duncan's Multiple Range Test at 5% level of significance. The data of the paired preference test were analysed using a 2-tailed binomial test at 5% level of significance. Analysis of data was done according to the methods of Watts et al (1989).

Results and Discussion

Processing : The average weight of a fresh bitter gourd was 196 g with approximately 80% being available for processing after removal of stem, seeds and endocarp. To reduce the bitterness, the bitter gourd rings were subjected to pre-treatments of soaking and /or blanching in NaCl solutions. Table 1 indicates that only two treatments (T3), which involved blanching fruit slices in 5% NaCl solution either at 100°C for 3 min (HTST) or using a two-stage blanching at 70-80°C for 20 min (LTLT) followed by 100°C for 3 min (HTST) yielded acceptable products, based on the removal of bitterness and maintenance of firmness (texture). The bitter gourd fruit contains an alkaloid momordicin, which is responsible for the bitterness of the fruit (Kalra et al. 1988). All other pre-treatments of bitter gourd resulted in products that were either bitter or had an undesirable texture. Blanching of fruits in 10% NaCl solution resulted in softer textured product in comparison to blanching in 5% NaCl solution. Seow et al (1991) reported that firmness of bitter gourd was greatly influenced by thermal pre-treatment of low temperatures as maximum firmness of bitter gourd slices was obtained, when LTLT pre-treatment was conducted at temperatures ranging from 60-80°C. Also, they reported a two to three-fold increase in firmness, when vegetables were LTLT blanched at their optimum temperatures. As reported, for other tissues, van Buren (1984), indicated that softening may be caused by the displacement of calcium in the tissue by sodium chloride. Also, in the process of osmosis, water moves from the cell containing water into the brine, resulting in a tendency to equalise water concentration inside and outside the cell, causing partial dehydration of the cell (Potter and Hotchkiss 1995).

Physico-chemical analysis : Pickled products stored up to 32 days at 30-32°C resulted in loss of flesh firmness (Fig. 2). Pre-treatments of low-temperature long-time (LTLT) blanching followed by high-temperature short-time (HTST) resulted in firmer products, when compared to HTST treatments. The addition of $CaCl_2$ (T3C; T6C) in the blanching solution enhanced the firmness of the pickled products. Low-temperature long -time (LTLT) blanching can improve the firmness of processed vegetables and fruits by activation of the native pectin methyl esterase (PME) in the tissue of these products (van Buren et al. 1960; Lin and Schyvens 1995). Pectin methyl esterase acts by de-esterifying pectic substances in the plant cell walls, thereby increasing the amount of free carboxyl groups, which are then able to form cross-links by

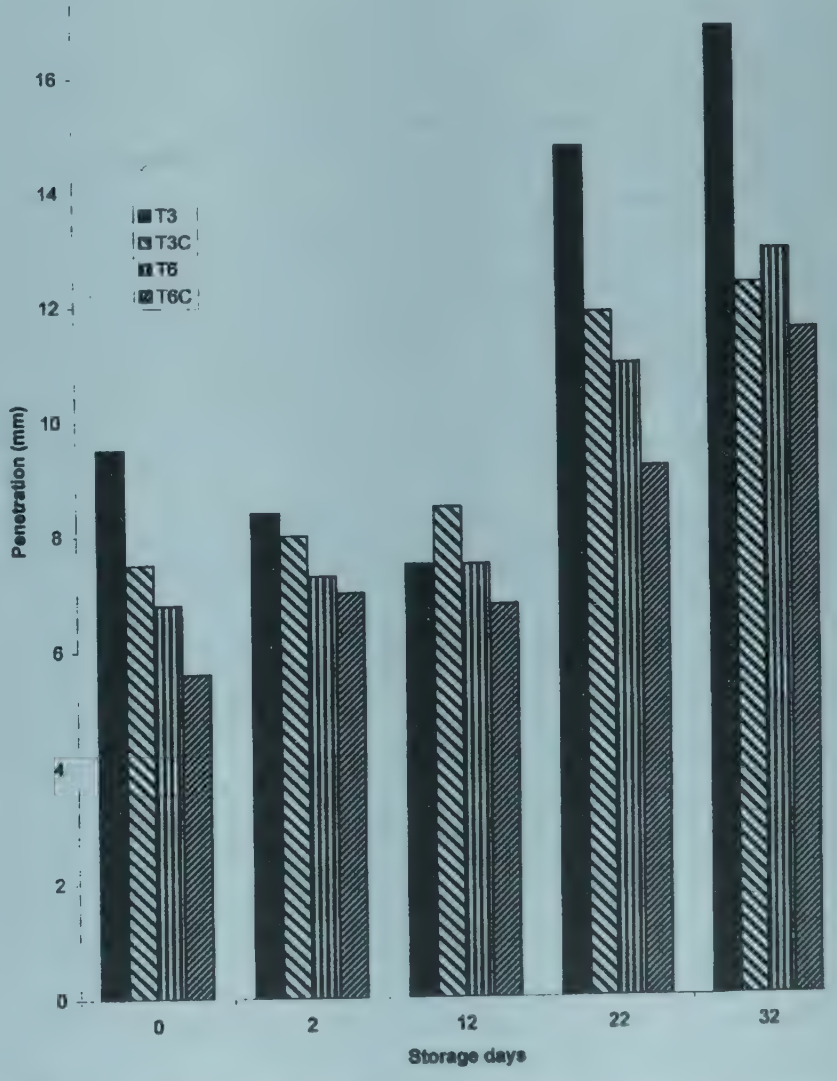

Fig. 2. Effect of different blanching treatments on texture of pickled bitter gourd during storage

salt-bridges formation with divalent anions (notably Ca^{++}) naturally present in the tissues or added to the blanch solution, thus firming up the cellular structure (van Buren et al 1960; Lee et al. 1979). Calcium pectates are water insoluble, when they are produced within tissues of fruits and vegetables (Potter and Hotchkiss 1995). The demethylation reaction is usually allowed to proceed for 15-30 min before PME and other enzymes are destroyed by a second blanch at higher temperatures in the two-stage step-wise blanching process (Seow et al. 1991). These researchers found that maximum firmness of bitter gourd slices was attained, when the LTLT pre-treatment was conducted at temperatures ranging from 60-80°C and that the increase in firmness was two-to three-fold, when vegetables were treated (LTLT) at their optimum temperatures.

Comparing the 'L' values of the whole raw fruits to those of the pickled fruits, the lower 'L' values recorded for the pickled products indicated a darkening of colour on processing (Table 3). On storage, further darkening of the processed product was indicated by the lower 'L' values on day 32. The lower negative 'a' values and higher positive 'b' values of the whole fruits when compared to values of the pickled products, reflected changes from green to dark yellow. Variations in a:b ratios reflected the discernible shifts in colour from dark green in the fruits to a yellow colour after pickling. Foda et al (1968) have stated that the retention of chlorophyll, which is the main pigment formed in the bitter gourd fruit is reduced on blacnhing and that the chlorophyll is converted to phaeophytin, which increases with time and temperature of blanching. However, Schwartz and Lorenzo (1990), reasoned that since phaeophytin conversion was dependent on an acid medium, the green colour of chlorophyll may be preserved by controlling the pH of the medium.

The pH values of the HTST blanched bitter gourd rings (Table 1; T3 and T3C) with or without the addition of 0.1% $CaCl_2$ were higher (5.2 ± 0.10) than the pH values recorded for those fruits (Table 1; T6 and T6C) blanched in the two-stage blanching process (4.9 ± 0.10). From day 2 to day 32 on pickling, all the products had attained pH values between 3.17-3.20.

There was greater loss of ascorbic acid in fruits blanched at the two-stage LTLT blanching process (Table 1; T6 and T6C with or without 0.1% $CaCl_2$ as compared to those blanched at HTST (Table 1; T3 and T3C with or without addition of 0.1% $CaCl_2$). The bitter gourd rings after HTST blanching had an average ascorbic acid content of 35.72 ± 0.15 mg/100 g fruit tissue, whereas fruits subjected to the two-stage blacnhing (LTLT and HTST) had an average ascorbic acid content of 17.86 ± 0.12 mg per 100 g fruit tissue. On day 2 after pickling, the ascorbic acid content in all treatments had dropped to 13.40 ± 0.10 mg/100 g fruit tissue and by day 12 on pickling, there was stabilisation of the ascorbic acid content to 4.47 ± 0.12 mg/100 g fruit tissue. The ascorbic acid of bitter gourd as reported by FAO (1972) was approximately 57 mg/ 100 g edible portion. Kalra et al (1983) analysed various cultivars of bittergourd and found ascorbic acid in the range of 96.3 - 144.1 mg/100 g of fresh tissue and that there was

TABLE 3. COLOUR OF FRUIT AND CHANGES IN COLOUR OF PICKLED BITTER GOURD ON STORAGE (30-32°C)

	Colour			
Fruit or pickle	L	a	b	a./b
Whole fruit	42.10	-16.27	30.10	-0.54
Raw fruit	41.17	-15.60	28.13	-0.56
Day 0 storage				
T3	37.03	-13.07	22.83	-.057
T3C	36.90	-13.20	23.97	-0.55
T6	33.00	-5.00	18.73	-0.27
T6C	38.30	-5.80	24.00	-0.24
Day 2 storage				
T3	33.99	-2.27	19.69	-0.16
T3C	33.62	-2.03	19.34	-0.11
T6	33.54	-2.34	17.63	-0.13
T6C	35.40	-2.70	18.62	-0.14
Day 12 storage				
T3	32.51	-2.70	17.28	-0.16
T3C	35.36	-2.56	17.94	-0.14
T6	33.54	-2.34	17.63	-0.13
T6C	32.68	-2.59	18.63	-0.14
Day 22 storage				
T3	32.93	-1.90	18.18	-0.10
T3C	33.97	-2.09	18.24	-0.11
T6	32.61	-2.20	16.66	-0.13
T6C	30.37	-1.30	15.42	-0.08
Day 32 storage				
T3	31.54	-1.71	16.40	-0.10
T3C	33.57	-1.80	16.86	-0.11
T6	32.71	-2.48	17.72	-0.14
T6C	33.88	-3.17	17.77	-0.18

T3-blanched in 5% NaCl solution at 100°C for 3 min (HTST)

T3C-blanched in 5% NaCl solution with 0.1% $CaCl_2$ at 100°C for 3 min

T6-blanched in 5% NaCl solution at 70-80°C for 20 min (LTLT) followed by 100°C for 3 min (HTST)

T6C-blanched in 5% NaCl solution at 70-80°C for 20 min with 0.1% $CaCl_2$ (LTLT) followed by 100°C for 3 min (HTST)

TABLE 4. EFFECT OF TREATMENTS ON SENSORY SCORES OF PICKLED BITTER GOURD

Storage days at 30-32°C	T3	T3C	T6	T^C
2	6.58[a]	7.08[a]	7.33[a]	7.83[a]
12	7.00[a]	7.00[a]	7.30[a]	7.83[a]
22	6.00[b]	6.67[b]	7.75[a]	8.00[a]
32	5.42[b]	7.17[a]	7.33[a]	8.17[a]

Any two means followed by different superscripts are significantly different at 5% level

Average of scores of 12 panelists (9-like extremely, 5-neither like nor dislike, 1-dislike extremely).

T3 - blanched in 5% NaCl solution at 100°C for 3 min (HTST)

T3C - blanched in 5% NaCl solution with 0.1% $CaCl_2$ at 100°C for 3 min (HTST)

T6 - blanched in 5% NaCl solution at 70-80°C for 20 min (LTLT) followed by 100°C for 3 min

T6C-blanched in 5% NaCl solution at 70-80°C for 20 min (LTLT) followed by 100°C for 3 min with 0.1% $CaCl_2$

a high percent loss of ascorbic acid in bitter gourds during blanching and processing (canning) and with a progressive increase in the loss of ascorbic acid during storage. The percent loss in ascorbic acid ranged from 32.7 to 41.4 during three months of storage and 43.9 to 56.9 during six months of storage. According to Lathrop and Leung (1980), the degradation of ascorbic acid during thermal processing operations is due to its instability to heat, light, metal catalysts, oxygen and its relatively high water solubility. The loss of ascorbic acid during cooking of bitter gourd may be reduced to a minimum, if the prepared fruit is immersed in boiling water (Kalra et al. 1988).

Microbial analysis : Microbial analysis of pickles stored at 30-32°C up to day 32 indicated no evidence of microbial growth. The low pH of 3.17-3.20 and the heat processing must have been adequate to prevent microbial activity. According to Khurdiya (1995), the main problem in pickles is the spoilage by either yeasts or moulds, since both can grow in the presence of acid. Blanchfield (1969), indicated that the acetic acid of the vinegar in the pickling solution was the main factor responsible for self-preservation of pickled products. The acetic acid can arrest both aerobic and anaerobic fermentation in pickled products (Swisher and Swisher 1963).

Sensory evaluation : Effects of treatments (HTST) and (LTST) were significant ($P<0.05$) on the acceptability of the pickled products. Treatment T6C in which the bitter gourd rings were blanched in 5% NaCl solution with $CaCl_2$ addition at 70-80°C for 20 min, followed by blanching at 100°C for 3 min was rated with highest organoleptic scores (7.83-8.17; liked moderately to liked very much) on all storage days (Table 4). Paired preference evaluation between the most acceptable product (T6C) and the commercial pickled product indicated 100% preference for the experimental product by the panelists. Panelists indicated that the experimental product had an attractive uniform appearance, appealing colour with a unique flavour and suggested utilisation as a snack or appetiser or as a garnish in prepared dishes.

Conclusion

Pickled bitter gourd rings, which were blanched in 5% NaCl solution at 70-80°C for 20 min (LTLT) and then at 100°C for 3 min (HTST) with 0.1% $CaCl_2$ were firmer than other treatments and were rated with the highest Hedonic scores of being liked moderately to liked very much. The pH and colour stabilised after day 2 of pickling and there was no evidence of microbial growth throughout the storage period.

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RESEARCH PAPER

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Testing of A Convection Type Cylindrical Dryer for Production of Instant Soy-*dosa* Mix

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A convection type cylindrical dryer developed was evaluated for drying of soy-cereal blended slurry to get instant soy-*dosa* mix. Blanched soybean splits (10 and 20%) were used in combinations with rice and blackgram. Three ingredients were mixed in given proportions, soaked for 4 h and ground using a wet grinder. The mix was then kept for about 20 h for its natural fermentation followed by drying. For drying, three temperatures were used viz. 40,50 and 60°C. The observations were recorded on moisture content of the drying product, temperature of the inlet and outlet air, velocity of the air with and without load on the dryer at time intervals of 3 h to 18 h. The physical properties of instant soy-*dosa* mix such as colour, water absorption index and moisture were determined for each of the considered combinations. A control was maintained with zero proportion of soybean for comparison of results. Results indicated that the instant soy-*dosa* mix could be successfully dried using the developed slurry dryer for a duration of 12 h. The product obtained was utilized for production of good quality instant soy-*dosa*. The drying temperature of 60°C and incorporation of soybean up to 10% were found to be optimum from the view point of consumer acceptability as well as the ease of preparaton of the end product.

Keywords : Drying, Soybean, Instant soy-*dosa* mix, Economic analysis.

Soybean contains 40% protein, which is almost twice that is present in the popularly consumed pulses in India. Soybeans are good sources of dietary fibre, minerals and vitamins. Fermentation has long been recognized as one of the oldest methods of preservation. Fermentation has multifarious advantages such as enhancing the shelf life of foods, adding therapeutic values, enhancing the nutritional value and eliminating heat resistant anti-nutritional factors such as phytates (Jha and Mishra 1986) and flatulent galactosides (Jha and Verma 1980). Among the pupular fermented foods of Indian origin, the *dosa* holds an important place in the diet of Indians especially from South. It is made with a combination of rice and blackgram in different proportions, most common being 2:1 or 3:1. The fermentation process usually adds desirable nutrients not present in the original raw material, such as vitamin B_{12}. Typically, fermentation does not greatly alter the amino acids in cereals and soybeans, but often increases the availability of proteins. Further, the undesirable beany flavour is destroyed when fermentation process is used for soybeans. The instant soy-fortified *dosa* material, when prepared will thus provide a better nutritional quality product to the consumers, with less preparation time and also will provide the advantages of fermented foods.

To make the instant product or *dosa* mix, it is necessary to dry the slurry such that it reconstitutes to the original state after water addition before actual *dosa* preparation. During drying of a wet solid with heated air, the air supplies the necessary heat and latent heat of vaporization to the moisture and also acts as a carrier for removal of the water vapour formed from the vicinity of the evaporating surface. The operation of slurry drying is generally continuous by contact heat transfer under vacuum or under atmospheric conditions. Normally, band or drum dryers are used where the liquid slurry paste is formed into sheet around the drum as the water is evaporated. The steps through which the slurry type materials undergo while drying are given in Fig. 1.

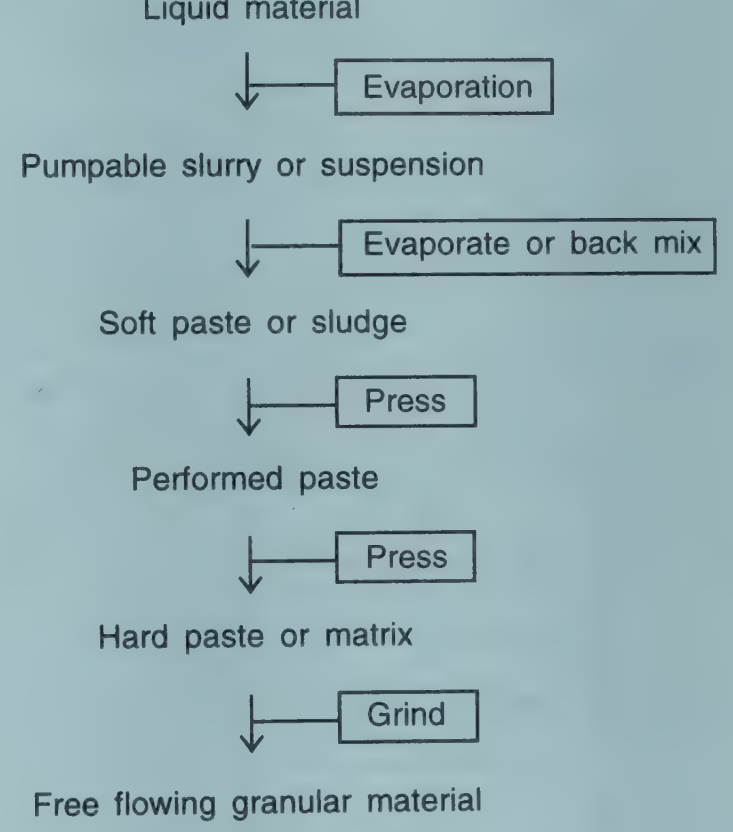

Fig. 1. General process flow chart for slurry drying by drum dryer

The unit operations are shown in the box and stage of material progress by arrow. The liquid material is first evaporated, when it is poured on the drum dryer. The heat for evaporation is supplied by the hot surface of the drum, which is generally heated by steam from inside the drum. In the next stage, the evaporation continues from the materials and the semi-dried material is mixed in the fresh slurry. The next stage follows pressing of the hot semi-dried material to

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squeeze out the water to the surface, so that it can be easily evaporated. The product obtained after this stage is a performed paste, which is further pressed to get hard paste or matrix. This material is then ground to get the desired particle size and better reconstitution of the material, when the water is added. This yields the free flowing granular material easy to pack in the pouches and can be preserved for a longer time. However, the drum dryers require boiler for steam generation and hence are generally expensive proposition. Hence, a low cost cylindrical dryer was designed at CIAE (Jaswant Singh and Jha 1992) for facilitating the drying of slurries. The objective of the present study was to test and evaluate the dryer for fermented soy slurry, estimate the cost of the dryer from its materials list and also to work out the economics of the drying to find out its economic feasibility.

Materials and Methods

Preparation of batter : The materials selected for drying were soy, rice and balckgram to get a final product of soy-fortified *dosa* mix. Three combinations of rice, blackgram and soybeans in the proportions of 20:10:0, 20:7:3 and 20:4:6 giving 0, 10 and 20% of blanched soybean *dhal* fortification in the instant *dosa* mix. All the ingredients were mixed in given proportions and soaked for 4 h. The soaked material was course ground in the domestic mixer. After thorough mixing, the contents were kept for natural fermentation for 20 h (overnight). Next day, the material was dried in the trays provided in a cylindrical dryer.

Cylindrical dryer : The dryer as shown in Fig. 2. consisted of three main parts. : 1) The cylindrical chamber (housed plenum as well as drying chamber). The central pipe shaft with perforations for air distribution. Exhaust air outlet provided with inverted funnel type cover for exit of saturated air. 2) A blower with 2 hp motor and 3) the 3 kW heaters to heat the drying air. The cylindrical chamber consisted of seven shelves, each fitted with three circular trays of 25 cm diameter and 2.5 cm depth supported by the central hollow shaft. Each tray was provided with the perforations and could hold the wet material of 400-450 g, thus giving the overall capacity of 8-9 kg per batch for 21 trays.

Dryer evaluation : The dryer was evaluated by recording the distribution pattern of drying air temperature and relative humidity during drying. The air temperature and RH distribution was recorded both for no load and under material drying conditions. The temperature of drying air was recorded by

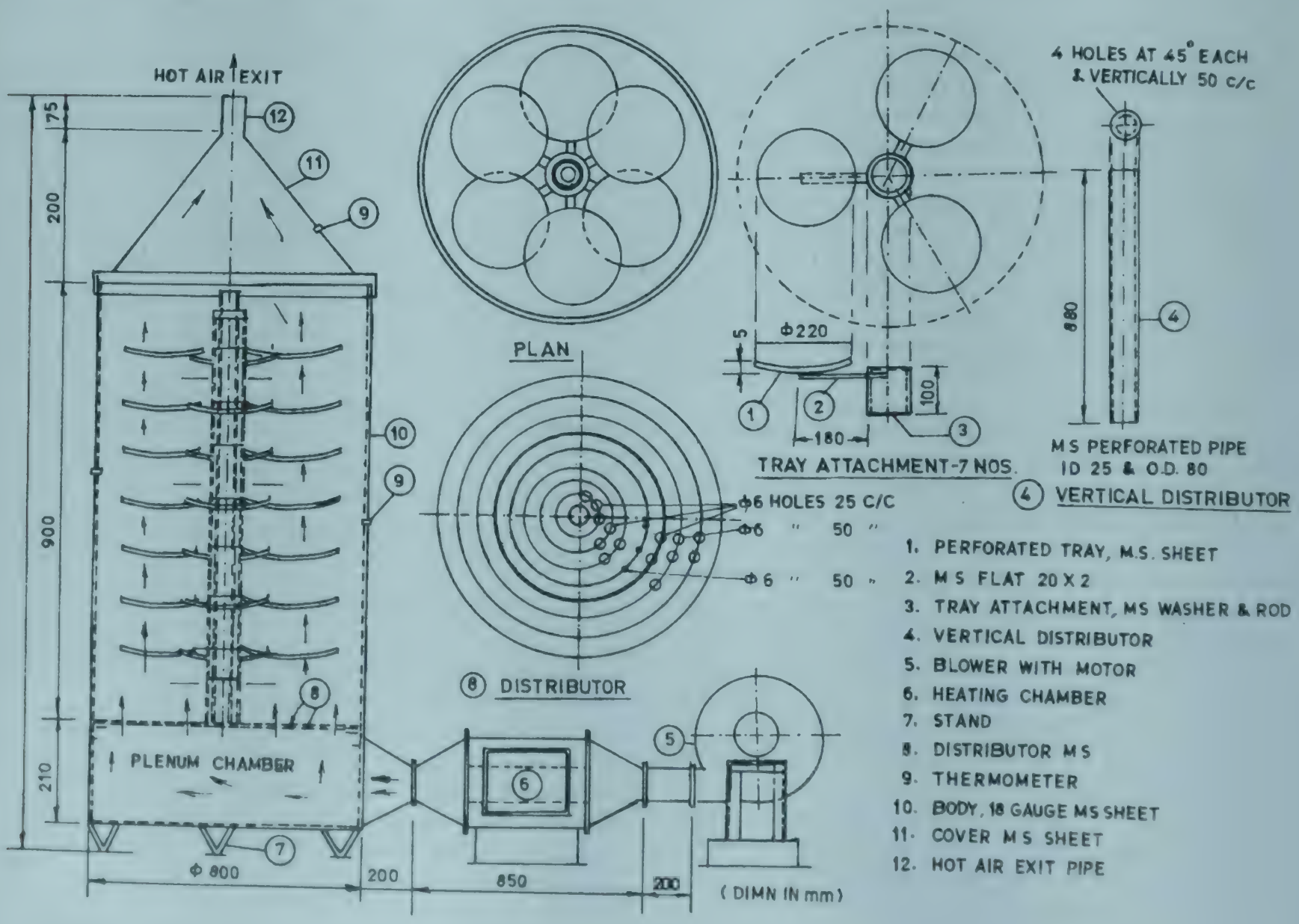

Fig. 2. CIAE slurry dryer

mercury thermometer for which the observation holes were provided at different heights of the dryer which were plugged when observations were not recorded. The air velocity in the dryer was measured with vane anemometer (Make: Ota Keiki Seisakusho, Japan). The moisture content of the slurry during drying was measured by drawing the samples randomly from the various trays. The AOAC (1975) method of 110°C for 12h was followed for sample size of 5 g. The moisture content was measured for each height at the interval of 3 h till the material was dried down to safe moisture content of 6%. The drying operation was conducted at three air temperatures of 40, 50 and 60°C.

Measurement of physical properties : The major physical properties, which get affected by the process of drying are colour, bulk density, hydration ratio and moisture content of the final material. The colour was determined by glass reflectance meter (Associate Instruments, New Delhi). Magnesium oxide (AR grade) was used as standard for complete whiteness (100% reflectance setting of the instrument). The observations were recorded in duplicate. The bulk density of the free flowing granular material was measured as tap density where the material was poured up to 20 ml mark in stages and for each filling, 10 taps were given. At the end of fifth tapping, the material was weighed. A measuring cylinder of 100 ml was used for the experiment (Patil et al. 1991). Water absorption capacity was measured by pouring about 50 g sample in a 500 ml beaker containing water. The mixture was stirred and allowed to stand for 30 min. At the end, the excess water was drained and the wet weight of the sample was measured. The increase in weight per unit initial weight expressed in percentage was the water absorption index.

Results and Discussion

Testing of dryer : The initial moisture content varied among the batters prepared in the range of 64.53% to 78.5% as shown in Table 1. However, the average moisture content was above 70% except for one set of experiment. The air flow rate was 58.2 m³/min ± 3.55. The drying curves for top as well as bottom trays of the dryer at all three temperatures are shown in Fig. 3 to 5. It can be seen that there was no appreciable difference in drying behaviour at the top as well

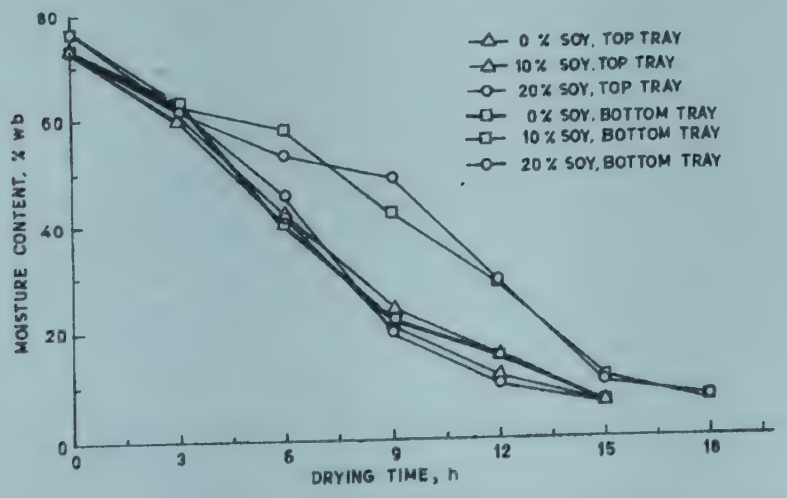

Fig. 3. Drying curves for soy batters at top and bottom trays at 40°C temperature

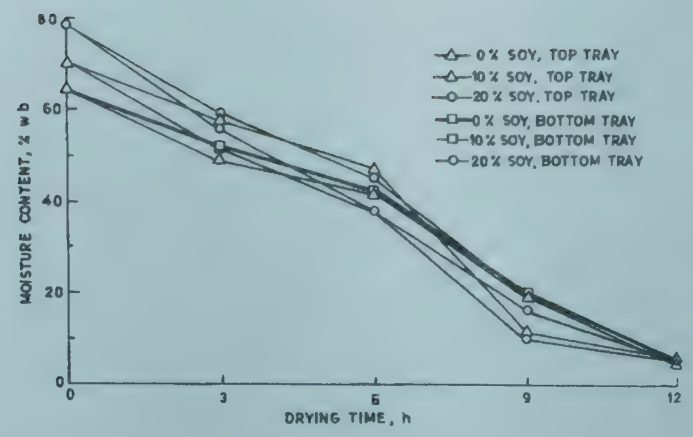

Fig. 4. Drying curves for soy batters at top and bottom trays at 50°C temperature

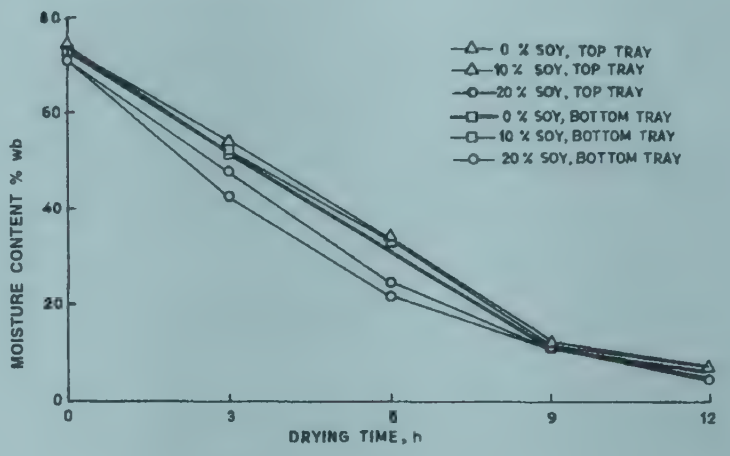

Fig. 5. Drying curves for soy batters at top and bottom trays at 60°C temperature

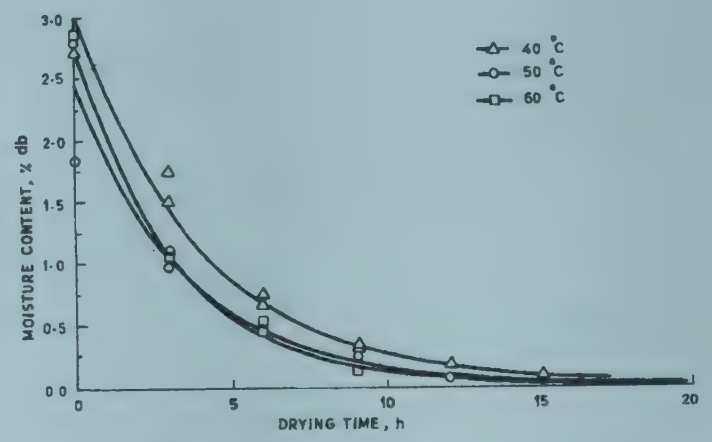

Fig. 6. Drying curves for 0% soy batters at different drying air temperature

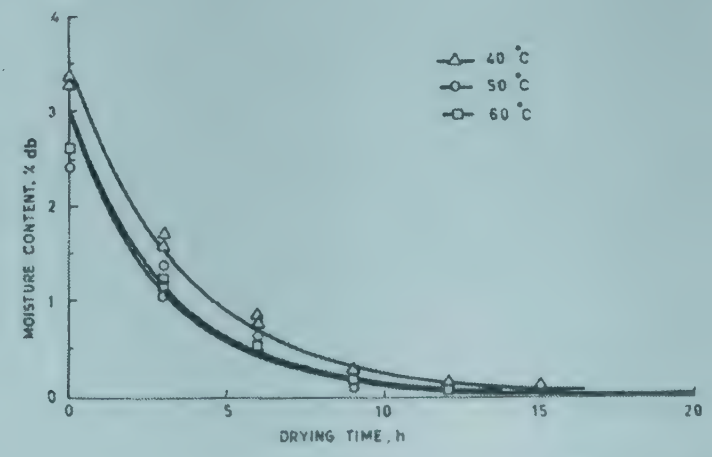

Fig 7. Drying curves for 10% soy batter at different drying air temperature

TABLE 1. PHYSICAL PROPERTIES OF THE DRIED MATERIAL

Soy %	Drying temp, C	Initial MC*	Bulk density*kg/m³	Hydration %@	Recovery at 6% moisture#	Colour %*
0	40	73.08 (1.13)	0.82 (0.02)	369.35 (2.89)	0.29 (0.012)	83.5 (0.707)
0	50	64.53 (0.14)	0.79 (0.03)	327.85 (4.74)	0.38 (0.001)	84.0 (1.414)
0	60	73.75 (0.32)	0.89 (0.04)	336.75 (6.43)	0.28 (0.003)	83.0 (1.414)
10	40	76.74 (0.33)	0.78 (0.03)	335.15 (1.48)	0.25 (0.004)	79.5 (0.707)
10	50	70.63 (0.17)	0.85 (0.03)	380.30 (12.58)	0.31 (0.002)	78.0 (0.000)
10	50	72.33 (0.17)	0.72 (0.03)	367.85 (7.14)	0.29 (0.002)	79.5 (0.707)
20	40	73.56 (1.37)	0.81 (0.01	429.80 (1.98)	0.28 (0.015)	76.0 (1.414)
20	50	78.50 (0.26)	0.73 (0.05)	442.75 (7.70)	0.23 (0.003)	75.5 (2.121)
20	60	70.92 (0.64)	0.77 (0.04)	452.25 (3.46)	0.31 (0.007)	77.0 (1.414)

The values in parentheses are standard deviations, + average of 6 observations, @ average of 4 replications, average of duplicate observations*.
@ temperature of drying air ± 2.3° C

TABLE 2. SUMMARY OF DRYER TEST RESULTS GIVING THE DRYING EQUATIONS AND PREDICTED DRYING TIME AT DIFFERENT DRYING AIR TEMPERATURES

% Soy blending	Drying air temp, C	Equation	R squared	Predicted timedrying at MC 0.05 db, h
0	40	$M = 3.04\ 10^{-0.1076t}$	0.99	16.58
0	50	$M = 2.45\ 10^{-0.1223t}$	0.92	13.82
0	60	$M = 2.81\ 10^{-0.1387t}$	0.98	12.62
10	40	$M = 2.81\ 10^{-0.1189t}$	0.99	15.49
10	50	$M = 2.81\ 10^{-0.1381t}$	0.95	12.86
10	50	$M = 2.81\ 10^{-0.1438t}$	0.98	12.31
20	40	$M = 2.81\ 10^{-0.0906t}$	0.95	20.49
20	50	$M = 2.81\ 10^{-0.1478t}$	0.98	12.97
20	60	$M = 2.81\ 10^{-0.1431t}$	0.99	11.95

TABLE 3. COST ESTIMATION OF THE DRYER FROM THE MATERIALS PROCURED

Item	Material	Quantity	Unit price, Rs	Cost, Rs
Blower, 2HP, 2000 rpm	-	1	10500	10500.00
Heating unit with 3000 W finned strip heaters	MS sheet 18 gauge	1	2000	2000.00
Ducting	MS sheet 18 gauge	12 sq ft	25/sq ft	300.00
Drying chamber				
Main body	MS sheet 18 gauge	24 sq ft	25/sq ft	600.00
Cover	MS sheet 18 gauge	5 sq ft	25/sq ft	125.00
Trays	Aluminum	12 sq ft	25/sq ft	300.00
Metal work	MS 3 kg	3 kg	30/kg	90.00
Electrical wiring	2.5 mm dia copper wire	48 ft	10/ft	480.00
Control box	LS	LS	LS	250.00
Thermostat	-	1	300	300.00
Nuts and bolts	-	25	2/each	50.00
Welding charges	-	40 ft	6/ft	240.00
Labour charges for fabrication	LS	LS	LS	700.00
			Total	15935 say 16000.00

TABLE 4. ECONOMICS OF SLURRY DRYER

Capacity of the dryer	3 kg powder/12 h
Cost of the dryer	Rs. 16000.00
Days of operation per year	250 days
Hours of operation per day	12 h
Manpower required	1
Cost of labour	Rs. 70.00
Life of machine :	10 years
Salvage value	Rs. 1600.00
Repair and maintenance charges	Rs. 1600.00
Interest @ 18%	Rs. 1440
Rate of sale of powder	Rs. 160.00/kg
Electricity used per batch	
a) Heater - 3*10 = 30 kWh	
b) Blower 2 HP = 1.5*10-15kWh	
c) Grinder = 1.5 kW*.25 = 0.375 kWh	
Total = 45.375 kWh	
Cost of electricity @ Rs. 2.5/kWh	Rs. 108.44
Depreciation	Rs. 1440
Cost of raw materials	
Rice = 2kg @ Rs. 15/kg	Rs. 30.00
Urad dal = 0.7 kg @ Rs. 24/kg	Rs. 16.8
Soy*dhal* = 0.3 kg @ Rs. 10/kg	Rs. 3.00
	Total 49.8 say Rs. 50.00
Cost operation per day	
Labour/day	Rs. 70.00
Repair and Maintenance/day	Rs. 6.4
Interest/day	Rs. 5.76
Electricity charges/day	Rs. 108.44
Depreciation/day	Rs. 5.76
Cost of raw materials/day	Rs. 50.00
Total cost per day	Rs. 246.50
Sale price/kg for dry powder	Rs. 160.00
Net profit per day	Rs. 234.00
Pay back period	0.23 years
Break even point	200 kg

The actual cost of finished product has been worked out to Rs. 246.50/3 = Rs. 82.2/kg

The cost of similar material available in the market came to about Rs. 160.00/kg

Hence, the profit per day came to 234 for 3 kg per day

as bottom tray, indicating that the drying air was uniformly distributed. The moisture content for these curves are represented on wet basis. Once the drying behaviour has been confirmed as uniform, the drying data from both the trays were used for analyzing the drying curves to find out the effect of drying air temperature on the drying rate constants. The moisture content versus the drying time for 0 and 10 % soy blending at different moisture contents are shown in Fig. 6 and 7. The moisture contents in these curves are expressed on dry basis for all temperatures and the mathematical relationship found best fitting was of the following form:

$$M = C\ 10^{-Dt} \qquad \text{.....(1)}$$

where M = moisture content at time in decimal dry basis, elapsed drying time in h, C and D = drying constants. The prediction equations with the values of constants and coefficient of determination are given in Table 2.

Economics of the dryer : The dryer offered an advantage of drying slurry type of material even at low cost, otherwise it would not be possible as the drum dryers were expensive and required accessories like boiler. The cost of the dryer was calculated from the bill of materials required for its fabrication as given in Table 3. The cost of the dryer was estimated at Rs. 16000.00.

It can be seen from the prediction drying equation that for reaching to 5% moisture content, time required was about 12 h at drying air temperature of 60°C, whereas at 50°C, the drying time required was about 13 h. However, at 40°C, the drying time required was 25% longer than required at 60°C.

To arrive at the conclusion for optimum drying time, the quality of the dried material is also very important. The physical properties of the dried powder measured by standard methods are given in Table 1. The most important quality parameter is reconstitution ability of the material. In this case, it is water absorption and making the material very similar to fresh fermented material when the water is added. It was found that hydration was best at 20% soy blending, when dried at 60°C. The recovery of the dried material in the powdered form the ranged from 23 to 38%, depending on the initial moisture content of the slurry. The initial moisture content varied, as there could not be exact control on water addition during grinding. However, this variable was not correlated with the soy blending or drying air temperature. Another important property was colour. Higher reflectance indicated whiter colour. It was observed that at 0 blending of soybean, the colour was whiter compared to creamish, when the 20% soy was blended. However, there was no significant difference in colour, when soy was blended either at 10 or 20%. The bulk density of dried granular material ranged from 720 kg/m^3 to 890 kg/m^3 average being 800 kg/m^3. There was no correlation between independent variable and bulk density.

The cost of drying was worked out by determining fixed cost and variable cost for drying one kg of powder. The details of the economic analysis are given in Table 4.

From the above simple calculations, it can be seen that the dryer is economically feasible to produce the instant *dosa* mix. Though the cost of production of per kg of mix comes to Rs. 82, the sale price of the product in the 250 g pack is quite high, i.e., about Rs. 160/kg, thus giving a profit of Rs. 234/day for the entrepreneur.

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RESEARCH PAPER

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An Improved Cooking Quality Test for Basmati Rice

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Pre-soaking of Basmati rice (15 to 60 min) before cooking in excess water reduced the time of cooking from 20 to 10 min and increased the dimensional changes due to cooking. Rice soaked for 30 min (I) and 45 min. (II) had higher elongation ratios (2.21 and 2.32, respectively) over the control unsoaked rice (1.82) upon soaking. The coefficient of dimensional changes (CDC, i.e., the ratio of increase in length to increase in breadth) increased from 8.4 for unsoaked control to 14.5 and 15.8 for I and II, respectively. The length to breadth ratio (L/B) of cooked basmati rice, (6.9 and 7.3 for I and II, respectively) was also higher than the unsoaked control (5.5). The loss of solids in gruel upon cooking (5 to 6%) remained more or less the same. Pre-soaking increased the dimensional changes in the non-basmati rices, 'Gujarat Parimal' (GP) and 'Haryana Gaurav' (HG) too, but the extent was less. Moreover, solids loss in gruel was higher in pre-soaked samples in these rices (10.6-12.7%). The differences in the CDC and L/B ratio of cooked rice can serve as parameters for differentiating Basmati from non-Basmati rice. Use of a multivalent inorganic compound during soaking helped to minimise kernel disintegration and longitudinal splitting during excess water cooking.

Keywords : Basmati rice, Cooking quality test, Cooking time, Pre-soaking, Sensory parameters.

Basmati, the 'Prince of rice' is a nature's gift to Indian sub-continent. The supremacy of Basmati rice over other scented varieties is attributed to its unique and well balanced combination of a number of characteristics such as aroma, long slender kernels, soft texture of the cooked rice, slight curvature with beaded appearance and a higher linear kernel elongation. These properties have elevated it to a unique status among all rice varieties. About a million tonnes of Basmati rice are produced annually in the country and about 60% of it is exported (Anon 1996; Siddiq 1996). The demand for Basmati in the USA is also reported to be increasing at the rate of 50% a year (Huke and Huke 1990).

Currently, the predominance of 'Taraori Basmati' in the local as well as export market is followed by 'Basmati-370' (also known as 'Punjab Basmati' or 'Amritsari' and Type-3 ('Dehraduni'. 'Taraori Basmati', evolved from Karnal local, commands the paramount position in export market (Mahindru 1995).

Progress in the trade is, however, facing new challenges due to lack of definite quality standards and adulteration with non-Basmati rice. Although several workers (Goyal and Pingale 1971, Khan and Ali 1985, Mahindru 1995, Nagaraju et al. 1975; Rani and Srinivasan 1989; Vaingankar and Kulkarni 1986; 1988; Vasudeva Singh et al. 1986; Whitworth et al. 1996) have attempted to differentiate Basmati from non-Basmati rice, literature on improvement of sensory properties of Basmati that could widen the difference in the cooked rice properties to use it as a possible tool for detection of adulteration is rather scanty (Mahindru 1995; Vaingankar and Kulkarni 1986).

This paper describes the results of studies undertaken to improve differentiative parameters of Basmati and non-Basmati upon cooking with a view to develop a package for quality control and up-gradation of Basmati rice in trade.

Materials and Methods

Basmati rice (1 kg consumer pack) of standard brands (names withheld, designated 'A' and 'B') were purchased from local market. Milled rice of 'Gujarat Parimal' (GP) and 'Haryana Gaurav' (HG) varieties received from traders for quality testing (which are reported as being commonly used as adulterants) were used for comparison with Basmati rice.

Procedure for determination of cooking time and sensory parameters : Milled rice was screened visually and whole sound grains were collected. Ten gram rice samples were soaked in 20 ml distilled water for 15, 30, 45 and 60 min in different flat bottom stainless steel wire mesh baskets (fabricated for the purpose) held in a 100 ml beaker at room temperature (25-28°C). Unsoaked (control) and soaked rice (held in wire mesh baskets) were then transferred to 250 ml beaker containing 150 ml slow boiling water over an electric stove (1.5 KW). Excess water from pre-soaking was also added to the boiling water. The cooking time was determined using Ranghino (1966) test, by withdrawing few grains periodically and pressing between two glass slides, till no opaque portion or white core remained.

At the end of cooking time, the wire-mesh basket along with rice was taken out and the excess water was transferred to a tared beaker. Cooked rice was washed with a little water with gentle shaking. This water was also added to the tared beaker for determining solids loss in cooking water (Batcher et al. 1956). The cooked grains were then spread in a 15 cm, dia. petri dish, covered with a lid and kept aside for determining the aroma and grain dimensions. Length and breadth before cooking (Lu and Bu, respectively), and after cooking (Lc and Bc, respectively) and length to breadth ratio of cooked rice (Lc/Bc), elongation ratio (ER, i.e, the ratio of the length of cooked grain to the length of raw grain), coefficient of dimensional changes (CDC, i.e, the ratio of increase in length to increase in breadth of the grain) were determined. (Juliano 1985 a, Mahindru 1995). A panel of 8 judges evaluated the aroma of the cooked rice by close sniffing, subjectively.

Measurement of length and breadth of rice : Ten whole grains (raw or cooked) were placed either lengthwise (with

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their respective ends, germ or distal) or breadth-wise, (with their respective sides, dorsal, ventral touching each other) on a flat plane surface along a millimeter scale. Cumulative length and breadth were noted and averaged. The measurements were repeated 20 times in each sample and thus an average of 200 grains was recorded (Juliano and Perez 1984).

Use of additives to reduce breakage of rice during cooking and improvement of sensory profile : Cooking in excess water led to disintegration of many grains limiting the availability of whole cooked grains for length and breadth measurements. In order that a maximum number of grains retain their integrity, experiments were carried out using various foodgrade chemicals as additives during soaking stage, selected on the basis of their ionic properties or interaction with organic polymers. They were tested at different concentrations in soaking water with 30 min soaking, using Basmati 'B' and 'HG' rice varieties.

Results and Discussion

Dimensional changes upon cooking : The physical parameters like length, breadth, elongation ratio and solid loss are presented in Table 1. It can be seen, as is also already known, that Basmati rice elongated more on cooking as compared to the non-Basmati rice. The length of rice in case of Basmati increased more than twice upon cooking after pre-soaking. These values were about 20 to 28% higher than for the rice, which was cooked directly without pre-soaking. Against this, the ER for GP (1.56) increased marginally by about 5% only by pre-soaking and cooking. Similarly, in the case of 'HG', which gave an 'ER' of 1.38 (very much lower than for the Basmati rice) showed about 15% higher value, when presoaked and cooked. On account of this, the relative difference between Basmati and non-Basmati was magnified as indicated by CDC, which ranged from 12.1 to 15.8 for Basmati as against about 3.0 to 4.6 for 'GP' and 4 for 'HG' varieties.

As is apparent, differences in the changes in dimension of grain upon cooking could serve as good indices for detecting adulteration. Although 'Lc/Bc' and 'ER' bring out the differences, the use of coefficient of dimensional changes in detection of Basmati rice adulteration has not been well documented. Mahindru (1995) has highlighted this parameter bringing out the difference between Basmati and an adulterant terrycot and concluding that CDC was a very important parameter. Present results substantiate this.

A higher extent of elongation of grains during cooking is one of the unique features of Basmati rice (Juliano 1985 b). Khan and Ali (1985) made comparative studies of Basmati 370 with non-Basmati rice and showed that ER, volume expansion ratio and water uptake ratio were high for the former. Jaliano (1985 a) has ascribed higher elongation of Basmati, rice to a preferential breakdown of the endosperm cell walls in a direction favouring elongation.

Length : breadth ratio upon cooking : The 'Lc/Bc' ratio of cooked Basmati was 7 for pre-soaked rice, as against 5.5 for unsoaked control. In case of 'GP', the values were 3.8 and 3.0 for pre-soaked and control, respectively. High 'Lc/Bc' ratio in cooked rice meant least breadth-wise swelling.

Cooking time : Pre-soaking of rice caused a reduction in the time required for cooking to about half in Basmati as well in 'HG' and 'GP' varieties (Table 1). Similar reduction in cooking time for Basmati after 30 min pre-soaking at room temperature (RT) has also been reported by Juliano (1985 a). Sowbhagya and Ali (1991) have also shown that pre-soaking of raw rice for 15 min at room temperature reduced the cooking time by half. They noted that pre-soaking caused an increase in the grain length by about 20% but a reduction in thickness by about 5%, in firmness by about 10% and in elasticity of the grain by about 25% over the unsoaked control. The decrease in the cooking time was speculated by them, to be due to the higher moisture content of pre-soaked rice facilitating faster heat transfer.

TABLE 1. EFFECT OF PRE-SOAKING OF RICE ON PHYSICAL AND SENSORY PROPERTIES

Rice	Presoaking time, min	Cooking time, min	Lu* mm	-Lc* mm	Bu* mm	Bc* mm	ER* (Lc/Lu)	Lc*/Bc	CDC*	Solids loss, %	Aroma of cooked rice**
Basmati A	Nil	20.0 ± 0.20	7.2 ± 0.12	13.1 ± 0.15	1.7 ± 0.08	2.4 ± 0.10	1.82	5.5	8.4	6.2 ± 0.08	++++
	15	10.0 ± 0.14		15.7 ± 0.15		2.4 ± 0.10	2.18	6.5	12.1	6.1 ± 0.08	+++
	30	10.0 ± 0.35		15.9 ± 0.15		2.3 ± 0.15	2.21	6.9	14.5	5.2 ± 0.08	++
	45	10.0 ± 0.54		16.7 ± 0.15		2.3 ± 0.11	2.32	7.3	15.8	5.5 ± 0.05	+
	60	9.0 ± 0.41		15.9 ± 0.08		2.4 ± 0.09	2.21	6.3	12.4	5.7 ± 0.08	+
'GP'	Nil	25.0 ± 0.41	6.4 ± 0.11	10.0 ± 0.16	1.8 ± 0.07	3.0 ± 0.07	1.56	3.3	3.0	7.0 ± 0.07	A
	15	15.0 ± 0.41		10.5 ± 0.05		2.7 ± 0.00	1.64	3.8	4.6	12.7 ± 0.09	A
	30	14.5 ± 0.41		10.5 ± 0.07		2.7 ± 0.07	1.64	3.8	4.6	10.6 ± 0.08	A
	45	14.0 ± 0.00		10.3 ± 0.06		2.7 ± 0.07	1.61	3.8	4.3	11.2 ± 0.08	A
	60	13.5 ± 0.41		11.5 ± 0.06		3.1 ± 0.06	1.79	3.7	3.9	12.2 ± 0.08	A
'HG'	Nil	23.0 ± 0.41	7.4 ± 0.70	10.2 ± 0.08	1.7 ± 0.07	2.6 ± 0.07	1.38	3.9	3.1	7.0 ± 0.07	++
	30	15.0 ± 0.41		11.9 ± 0.05		2.8 ± 0.09	1.61	4.2	4.1	10.6 ± 0.08	+

*Lu = Length of uncooked rice; Bu = Breadth of uncooked rice, Lc = Length of cooked rice; Bc = Breadth of cooked rice;

*ER = Elongation ratio; * CDC = Coefficient of dimensional changes; **++++ = Very strong; +++ = Strong; ++ = Moderate; + = Present; A = Absent

Solids loss in cooking water : The loss of solids in cooking water generally serves as a good parameter for the assessment of cooking quality of rice. The stickiness on cooking of rice is attributed to its tendency to yield a thick viscous gruel (Bhashyam 1976). It was observed that Basmati rice gave thin gruel as compared to non-Basmati rice (Vaingankar and Kulkarni 1986). In the present studies, the data (Table 1) showed that Basmati *per se* lost lesser solids in cooking water (5.2 to 6.2% only), irrespective of pre-soaking time. Against this, the non-Basmati 'HG' variety showed a slightly higher values of solid loss (7%), which increased further upon pre-soaking and cooking (10.6%). Variety 'GP' gave a higher solid loss (12.2%). This trend increased with increase in soaking time. It appears that, during soaking, kernels of non-Basmati rices suffer cracking, the cell contents become rather loose and leach out easily during cooking, resulting in a higher solid loss. It has been reported that cell walls of Basmati are more compact as compared to those in non-Basmati rice (Beerh and Srinivas 1991). However, it has also been reported (Desikachar and Subrahmanyan 1959), that solid loss upon cooking in excess water decreases with aging. This parameter may, therefore, not be a reliable indicator for difference between Basmati and non-Basmati rice varieties.

Aroma of cooked rice : The present study showed that pre-soaking prior to cooking caused a decrease in the aroma of cooked Basmati rice. It may be borne in mind that the rice was cooked in excess water and the gruel was separated out from the grains. The majority of the aroma, therefore, might have gone along with the cooking water, resulting in a decrease in the aroma of cooked rice. Against this, it was interesting to note that the characteristic aroma of non-Basmati, normal cooked rice was fully lost. The retention of aroma in Basmati rice even after pre-soaking, therefore, would be of help in differentiating it from the adulterant variety.

It may be noted here that, in practice, during preparation of various recipes in which Basmati rice is used, a right quantity of water is generally taken for soaking, which is then fully utilized during preparation of the finished product. This helps in retaining the aroma considerably.

Effect of additives vis-a-vis grain breakage during test cooking : One difficulty in testing the dimensional changes after cooking was the tendency of cooked grains to disintegrate. Addition of various chemicals during soaking (prior to cooking), was explored to improve its sensory properties and to reduce breakage during cooking. Among the 8 types of various additives tested, a multivalent inorganic compound (viz. tricalcium phosphate) at 0.05% level, reduced the breakage of cooked rice very effectively, while retaining the aroma and other dimensional characteristics (Table 2). Breakage was very negligible in both Basmati and the adulterant, facilitating easy, quick and confident separation of the adulterant. Except this particular chemical, other studies did not yield the desirable results.

TABLE 2. EFFECT OF ADDITIVE DURING SOAKING ON PHYSICAL AND SENSORY PROPERTIES AND BREAKAGE OF COOKED RICE

Parameter*	Without additive		With additive **	
	Basmati 'B'	'HG'	Basmati 'B'	'HG'
Lu, mm	7.70 ± 0.08	7.40 ± 0.07		
Bu, mm	1.70 ± 0.09	1.70 ± 0.06		
L/B	4.45	4.34		
Lc, mm	16.40 ± 0.08	11.90 ± 0.05	16.10 ± 0.15	11.78 ± 0.08
Bc, mm	2.60 ± 0.11	2.80 ± 0.09	2.50 ± 0.12	2.00 ± 0.08
Lc/Lu (ER)	2.10	1.60	2.10	1.60
Lc/Bc	6.43	4.20	6.40	4.10
CDC	10.20	4.10	10.40	3.80
Breakage in cooked rice, %	12.00 ± 0.81	10.00 ± 0.82	3.00 ± 0.81	2.00 ± 0.81
Aroma	+++	+	+++	+

* See footnote of Table 1 for details
** Tricalcium phosphate

Conclusion

Pre-soaking of Basmati rice for 30 min at room temperature (RT) followed by cooking in excess water enhanced the elongation and dimensional changes of kernels, rendering it easier to differentiate cooked Basmati from non-Basmati rices. Co-efficient of dimensional changes (CDC, i.e, the ratio of increase in length to the increase in breadth) was a better index of dimensional changes. Staining test developed by this laboratory earlier (Bhashyam et al. 1993), followed in combination with the CDC, could, therefore, serve as a reliable parameter to identify adulteration/purity of Basmati rice. Addition of a multivalent inorganic compound during soaking greatly helped in retention of the integrity of cooked kernels and thus facilitated easier measurement of the dimensional changes.

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RESEARCH PAPER

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Antinutrient Profile and Chemical Composition of Custard Powder Produced in Nigeria

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Four brands of custard powder, coded GD, RK, MS and DS were analysed for the contents of oxalate, hydrocyanate, tannic and phytic acids. The amounts of proteins, phosphorus, calcium, zinc, iron and vitamin C in these products were also determined. The concentration (mg/100g) ranges, were: oxalate 20-22; hydrocyanate, 10-11; tannic acid, 4-6; phytic acid, 0.2-0.3; phosphorus, 41-119; calcium, 15-164; zinc, 0.4-0.5 and iron, 2-11. The levels of mineral nutrients were generally higher in the fortified brands than the non-fortified custard powder. Vitamin C (11-17 mg/100 g) was only detected in the fortified brands of this product. The protein contents ranged between 0.5 and 0.6%. The percentage contribution of each brand of custard powder to the recommended dietary allowance (RDA) for most of the nutrients analysed was low. Against the backdrop of increasing popularity of custard powder as a weaning food, it has been suggested that there is a need for the compulsory fortification of this product as a strategy to combat nutrient deficiency in consumers.

Keywords : Antinutrients, Custard powder, Mineral elements, Fortification, Vitamin C

Maize is the third most cultivated cereal crop in Nigeria (Oyenuga 1968). The projected agricultural supply of this grain in the country for the year 1991 was 0.896 million metric tonnes, whereas the corresponding demand was 1.041 million metric tonnes (Titilola and Igben 1986). This has been attributed to increased industrial utilization and population growth. One important industrial procedure is the conversion of maize into starch, which is widely marketed in Nigeria as custard powder. Several brands of this product are available in the country as well as neighbouring African States. Custard powder is consumed by Nigerians as a breakfast meal and the product is gaining popularity as a weaning food. A careful inspection of the product labels on the various brands of custard powder reveals that most of them do not supply sufficient information concern-ing their nutritive value. This, notwithstanding, the custard powders produced in Nigeria can be broadly classified into two groups i.e. those fortified with essential nutrients and those non-fortified brands. As regards cost, the fortified brands of custard powder are more expensive than their non-fortied counterpart and for economic reasons, consumers generally prefer the less expensive brands.

The present study was carried out to generate information on the levels of proteins, calcium, iron, phosphorus, zinc and vitamin C in selected brands of custard powder produced in Nigeria. The antinutrient profile of these powders was also assessed in view of the reported effects of antinutrients on mineral metabolism.

Materials and Methods

Four different brands of custard powder (three samples of each brand) were purchased on different days from locations in the North (Jos), West (Lagos) and South Eastern Nigeria (Uyo). The samples were stored at room temperature in the Central Research Laboratory, University of Uyo, Uyo, Nigeria, where all the analyses were carried out. Based on the labelling information, the brand coded GD was fortified with phosphorus, calcium, iron and vitamin C. The branch coded RK was reported to have been fortified with iron and essential vitamins. The products coded MS and DS represented the non-fortified brands of custard powder.

Total oxalates were determined by the method of Dye (1956). In this procedure, 2.0 g of each sample was digested with dilute hydrochloric acid for 4 h at 50°C. The oxalate was precipitated from solution as calcium oxalate by treatment with a dilute solution of calcium chloride at 90°C. The precipitate was solubilised with hot dilute H_2SO_4 and titrated against 0.05M $KMn0_4$. The oxalate content was calculated according to the principles of volumetric analysis (1ml of 0.05 M $KMn0_4$ is equivalent to 2.2 mg oxalate). The alkaline titration method (AOAC 1984) was used in the determination of hydrogen cyanide. A 10 g portion of each sample was soaked for 4 h in distilled water. The suspension was subsequently steam-distilled into dilute NaOH solution. The distillate was treated with KI and titrated against 0.02 M $AgN0_3$ solution to a faint but permanently turbid end point. Every 1 ml of $AgN0_3$ is equivalent to 1.08 mg HCN. Phytic acid was determined by the method of Wheeler and Ferrel (1971). Custard powder (10 g) was extracted with 3% trichloroacetic acid. The extract was treated with $FeCl_3$ solution and the iron content of the precipitated ferric phytate was determined by atomic absorption spectrophotometry. A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content. Tannic acid was determined by following the procedure of Burns (1971). A 10 g portion of the sample was extracted into methanol. An aliquot of the filtered extract was treated with vanillin-HCl solution. The absorbance of this solution was measured at 500 nm. The concentration of tannin in the custard powder was determined by reference to a calibration curve prepared from standard solutions of catechin in methanol. Vitamin C (ascorbic acid) was estimated by the method of Scharrfert and Kingsley (1955) in which 2 g of the sample was extracted with 0.5%

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TABLE 1. OXALATE, HYDROCYANATE, TANNIC AND PHYTIC ACID CONTENTS OF CUSTARD POWDER*

Brand (Code)	Total oxalates, mg/100g	Hydrocyanate, mg/100g	Tannic acid, mg/100g	Phytic acid, mg/100g
Fortified				
GD	20.46 ± 1.80	11.52 ± 2.54	5.45 ± 2.80	0.22 ± 0.02
RK	22.00 ± 2.20	11.52 ± 2.54	4.14 ± 2.24	0.31 ± 0.05
Non-fortified				
MS	20.90 ± 1.10	10.08 ± 2.50	6.24 ± 1.86	0.25 ± 0.06
DS	21.27 ± 2.54	11.52 ± 2.54	5.08 ± 1.39	0.34 ± 0.09

* Each value represents the mean ± SD for triplicate determinations

oxalic acid. The ascorbic acid was oxidized and reacted with 2, 4-dinitrophenyl-hydrazine to form a hydrazone, whose absorbance was read at 515 nm. The amount of ascorbic acid (vitamin C) was determined by reference to a calibration curve prepared with standard ascorbic acid. Protein (N × 6.25) was determined by following the AOAC (1984) procedure.

Phosphorus was determined by the molybdovanadate colorimetric method (Vogel 1969) in which 1.5 g of the sample was ashed. The ash was treated with concentrated $HCl/HN0_3$ and filtered. The filtrate was treated with molybdovanadate reagent to develop a pink coloured complex, which was quantified at 440 nm. The concentration of phosphorus in the sample was read from a calibration curve prepared by using KH_2PO_4. Calcium, iron and zinc were determined using the atomic absorption spectrophotometer (Model AA 919, Pye Unicam, UK). All determinations were carried out in triplicate.

Results and Discussion

The antinutrient profiles of the various brands of custard powder are shown in Table 1. The value for total oxalate ranged between 20.46 and 22.00 mg/100 g. Estimates have shown that 20-40% of total oxalate is present in the soluble form, which may inhibit the absorption of divalent cations (Passmore and Eastwood 1986). The lethal dose of oxalate has been reported to range between 2 and 5% (Munro and Basir 1969). Thus, the levels of oxalate found in the custard powder are far below the threshold levels of oxalate toxicity. The amount of hydrogen cyanide in custard powder ranged between 10.08 and 11.52 mg/100 g. These values fall below the lethal dose of 35 mg/100 g reported by Oke (1969). The toxicity of hydrogen cyanide is attributed to its ability to inhibit the respiratory chain at the level of cytochrome oxidase (Lehinger 1982). The composition of phytic acid in the custard powders was low, ranging between 0.22 and 0.34 mg/100 g. Low levels of phytic acid are desirable in foods because high dietary phytate has been reported to exert a negative effect on the absorption of mineral nutrients (Hallberg et al. 1987; Sandberg 1990; Heaney et al. 1991). Slightly high levels of tannic acid (4.14 to 6.24 mg/100 g) were obtained in this study. Tannic acid has the potential for complex formation not only with dietary proteins but also with digestive enzymes, thereby, reducing the digestibility of proteins in food (Singh and Eggum 1984). Table 1 also shows that the levels of antinutrients were similar in both fortified and non-fortified brands of custard powder.

Table 2 shows the proteins, phosphorus, calcium zinc, iron and vitamin C contents of custard powder. The level of protein in the sample was low (0.5-0.6%). Maize, the major raw material for the production of custard powder, contains 10-13% proteins (Antai and Nzeribe 1992). Apparently, much of the protein is lost during the conversion of maize into custard powder. Vitamin C was only detected in the fortified brands of custard powder. Apart from its functions as an antixodant (Stocker and Frei 1994), it is also known to facilitate the absorption of iron (Passmore and Eastwood 1986). Hence, there is the need for the fortification of custard powder with vitamin C.

The mineral elements constitute an important group of nutrients required by the human body for optimal functions (WHO 1996). Calcium and phosphorus interact in the formation of bones. Significant amounts of these two elements are present in the fortified brands of custard powder analysed in this study. Zinc is a component of several metallo-enzymes, including the antioxidant enzyme superoxide dismutase, whose function is to catalyse the destruction of the superoxide radical (0_2) (Schaefer et al. 1995). Zinc deficiency has been found to accompany protein energy malnutrition (Sandstead and Smith 1996).

Unfortunately, low levels of zinc were detected in the various brands of custard powder. Conversely, beneficial amounts of iron were detected in the fortified brands of custard powder. The provision of adequate iron to the body

TABLE 2. PROTEINS, ASCORBIC ACID, PHOSPHORUS, CALCIUM, IRON, ZINC AND THE CALCULATED PHYTATE: ZINC MOLAR RATIOS IN CUSTARD POWDER

Brand (Code)	Protein*, g/100g	Ascorbic acid*, mg/100g	Phosphorus*, mg/100g	Calcium*, mg/100g	Iron*, mg/100g	Zinc*, mg/100g	Phytate/ Zinc
Fortified							
GD	0.66 ± 0.05	11.80 ± 2.36	119.67 ± 3.51^c	164.02 ± 0.39^b	9.00 ± 1.80^a	0.43 ± 0.03	0.05
RK	0.50 ± 0.06	17.07 ± 4.40	41.33 ± 4.16^b	15.11 ± 1.68^a	11.50 ± 2.78^a	0.50 ± 0.07	0.06
Non-fortified							
MS	0.53 ± 0.04	ND	59.67 ± 4.51^a	18.55 ± 0.39^a	3.61 ± 1.47^b	0.40 ± 0.04	0.06
DS	0.58 ± 0.06	ND	55.55 ± 2.08^a	17.91 ± 0.53^a	2.40 ± 1.39^b	0.51 ± 0.10	0.06

* Values represent the mean ± SD for triplicate determinations

$^{a-c}$ Means within each column followed by the same alphabet are not significantly different ($P<0.05$) by Duncan's multiple range test

ND = Not detected

is essential for balanced nutrition. Despite its essentiality, Lazkowsky (1985) observed that a deficiency of iron was the most common cause of anaemia in the paediatric population. In addition to reduced work efficiency, iron deficient children manifest a reduction in the rate of normal cognitive (intellectual) development (Pollit 1993).

An often quoted disadvantage inherent in the use of cereal-based foods as vehicles for food fortification is the interfering role of phytates on mineral element utilization (Hallberg et al. 1987; Heaney et al. 1991; Sandberg et al. 1996). In the present study, low levels of phytic acid in custard powder were observed. Turnlund et al (1984) demonstrated that phytate: zinc molar ratio of 15:1 might lead to reduced zinc bioavailability from the diet. However, the ratios of phytate: zinc obtained in this study (Table 2) indicate that phytate is not likely to hinder the bioavailability of zinc from custard powder.

During the preparation of a single meal of custard, about two spoonfuls of the powder are usually mixed in hot water to produce a porridge. The percentage contribution of this meal to the RDA for various nutrients in infants is shown in Table 3, which shows that as a consequence of fortification, there was considerable enhancement in the contribution of a meal of custard powder to the RDA for specific nutrients. Table 3 also reveals that apart from iron, the present level of fortification of custard power did not contribute substantially to the RDA of the fortified nutrient. Hence, the manufacturers may need to widen the scope and increase the magnitude of fortification. This, notwithstanding, the practice of adding milk (Igbedioh and Akinyele 1983) to the meal and/or the consumption of the meal with bread (Akapo and Olowu 1992) may improve the contribution of the meal to the RDA for specific nutrients.

The weaning period has been described as the period of transition from an exclusively milk diet to the complete range of foods taken by the adult (Jellife 1973). In Nigeria and the world over, traditional weaning foods are based on local starchy staples, usually, fermented cereals that are processed into powder and eaten after boiling into an aqueous paste (porridge) (Akinrele and Bassir 1967; Muller 1970). Custard powder is also used as a weaning food in Nigeria. The procedure for converting the powder into a porridge is simple and similar to that used in preparing other local weaning staples.

The major nutritional goal during the weaning period is the provision of balanced foods to the infant. Failure in this objective could result in various grades of protein energy malnutrition, which is estimated to affect up to half of the children in developing countries (Simeon and Grantham-McGregor 1990). According to Latham (1997), the term protein energy malnutrition describes a broad array of clinical conditions ranging from mild to serious. At one end of the spectrum, mild protein energy malnutrition manifests mainly as poor physical growth in children, while at the other end of the spectrum kwashiorkor, alongside nutritional marasmus have high case fatality rates. Apart from protein deficiency, there is mounting

TABLE 3. THE PERCENTAGE CONTRIBUTION OF EACH BRAND OF CUSTARD POWDER TO THE RDA OF VARIOUS NUTRIENTS*

Brand (Code)	Proteins	Ascorbic acid	Phosphorus	Calcium	Iron	Zinc
Fortified						
GD	0.29	4.72	2.39	3.28	14.40	0.69
RK	0.22	6.83	0.83	0.30	18.40	0.80
Non-fortified						
MS	0.24	-	1.19	0.37	5.78	0.64
DS	0.26	-	1.11	0.36	3.84	0.82

* Estimates are based on the use of two tablespoonful of custard powder (16 g) in the preparation of a single meal

evidence concerning the involvement of free radicals in the pathogenesis of kwashiorkor (Golden 1985; Golden and Ramdath 1987; Golden et al. 1991; Becker et al. 1994; 1995). Apparently, weaning diets containing an appropriate combination of proteins, energy, minerals and antioxidant scavengers (Diplock 1991) are best suited for the fight against protein energy malnutrition.

Conclusion

The present study has shown custard powder lacks the qualities of a ideal weaning food. For the product to perform this role, there is need for compulsory fortification with a balanced range of nutrients. Alternatively, the nutrient composition of custard powder could be improved by supplementation with legumes, such as soybean, which are reported to be rich in proteins and other nutrients that are deficient in custard.

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Extension and Prediction of Shelf Life of *Dudh Churpi*

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The effects of storage of *dudh churpi*, incorporated with or without potassium sorbate (0.1%, w/w) packed in screw capped glass bottles, high density polyethylene screw capped bottles, thermally sealed low density polyethylene pouches or left unpacked under ambient environments (6-32°C, 44-88% relative humidity) over a period of six months on moisture, titratable acidity, free fatty acids, tyrosine, hydroxymethylfurfural, reflectance and sensory attributes were studied. The physico-chemical and sensory qualities of unpacked samples deteriorated at a faster rate as compared to packed ones. Among the packages, glass bottle provided maximum protection against deterioration. The physico-chemical parameters fitted well to the second degree equation ($y=A+Bt+Ct^2$) with correlation coefficients >0.94. Using regression equation based on mean overall sensory acceptability scores and storage period, it was possible to predict the shelf life of *dudh churpi*. The predicted shelf life periods of sorbate added *dudh churpi* samples, stored in glass bottles, plastic bottles and polyethylene pouches were 871, 577 and 444 days, respectively.

Keywords : *Dudh churpi*, Potassium sorbate, Packaging, Physico-chemical attributes, Sensory scores.

Dudh churpi is a traditional milk product, widely manufactured on a cottage scale in some parts of the Indian subcontinent. Since the manufacture of *dudh churpi* is still confined to individual households, wide variations are encountered in its manufacturing practices, which, in turn, influence its quality attributes (Hossain et al. 1999 a). The application of combined hurdle technology extends the shelf stability of the food product (Leistner 1992). Shelf life of *dudh churpi* may be extended by the application of combined hurdle technology i.e., acid coagulation, cooking (heating), addition of sugar, smoking and drying to get a product with low moisture content. Although the product keeps well for a long period without refrigeration, it is susceptible to water adsorption and microbial contamination, resulting in decreased acceptability and shelf life. Therefore, an adequate packaging is essential for a safe and easy marketing and for retention of its natural characteristics till consumed or used otherwise. Sorbic acid and its salts are considered effective antimicrobial agents (Liewen and Marth 1985) and classified as GRAS (generally recognized as safe) additives for food preservation (Sen and Rajorhia 1997). The present investigation was undertaken to study the effect of sorbate and different packaging materials on storability of *dudh churpi* and to predict shelf life of the product.

Materials and Methods

Preparation of dudh churpi : Fresh cow's milk, procured from the Himalayan Co-operative Milk Producers' Union Limited (HIMUL) in Matigara, was standardized to 1.0% (w/w) fat and 8.7% (w/w) solids-not-fat (SNF), followed by heating to 70°C, coagulating the milk within 60 sec with hot (70°C) 2.0%(w/v) citric acid solution, removing whey by instant filtering through a muslin cloth, cooking the coagulum in a stainless steel container over hot water bath and pressing the cooked coagulum at 9 kg cm^{-2} for 12 h (Pal et al. 1996). The pressed coagulum was cut into pieces of equal sizes (4 cm × 2 cm × 2 cm), smoked for 30 min with wood charcoal and dried in a hot air oven at 35°C to a moisture level of 30% (w/w). The partially dried *churpi* (pre*churpi*) pieces were then cooked for 15 min in concentrated milk, containing (w/w) 2.5% fat, 21.7% SNF and 5.0% sucrose in a stainless steel vessel, which was partially closed to reduce water evaporation. The contents were cooled to room temperature and the cooked pre*churpi* blocks were dried similarly for 15-20 days to a moisture content of approximately 15% (w/w) (Hossain et al. 1998, 1999 b, 1999 c).

One lot of sample was prepared by incorporating 0.1% (w/w) potassium sorbate (HiMedia, Mumbai, India) in concentrated milk at the time of cooking *prechurpi* (A). The other lot was taken as a negative control (B). The packaging materials, procured from Arihant Plastics Pvt Ltd, Calcutta and used for storage study, were 200 ml-screw capped glass bottles (P_2), 300μm-high density polyethylene bottles (P_3), and 150 μm-low density polyethylene film-made pouches (P_4). The control samples (P_1) were not packed, but kept uncovered on plastic trays.

Inner portions of the packages were cleaned with a detergent solution, chemically sterilized by treating for 5 min with 0.5% (v/v) hydrogen peroxide solution and then air dried. The whole operation was carried out in an aseptic condition.

A 200 g sample was packaged in each container and the sealed packages as well as unpackaged samples were stored at ambient temperature (6-32°C, RH 44-88%). The samples were subjected to sensory and physico-chemical analyses at an interval of 30 days.

Chemical analysis : The samples of *dudh churpi* were cut into smaller pieces and ground to a homogenous mass using an electric grinder. The ground mass was analyzed for moisture and titratable acidity (AOAC 1990), fat (ISI 1981), proteins and sugar (ISI 1967), ash (ISI 1980), free fatty acids (FFA) (ISI 1966), 2-thiobarbituric acid (TBA) value (Sidwell et al. 1954), tyrosine (Hull 1947), hydroxymethylfurfural (HMF) (Keeney and Bassette 1959) and reflectance using type CL-28 Elico reflectometer and a magnesium block, which gave 100% reflectance of 450 nm light.

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Sensory evaluation : Dudh churpi samples were evaluated at regular intervals of 30 days by a panel of 7 trained judges for overall sensory quality consisting of flavour (10), body and texture (10), and colour and appearance (5), using a 25-point score card, specially developed for the purpose (Hossain et al. 1996). All evaluations were conducted in quadruplicates.

Data analysis : Data on physico-chemical and sensory attribute parameters were analyzed by a 3-way factorial design (Snedecor and Cochran 1989).

The accuracy of fit of regression equations for overall acceptability as related to shelf life of *dudh churpi* was checked by calculating the root mean square (RMS) percent error.

$$\% \text{ RMS} = \sqrt{\frac{1}{n}\sum_{1}^{n}\left(\frac{t_{exp} - t_{cal}}{t_{exp}}\right)^2} \times 100$$

where t_{exp} = an experimental value for storage period and t_{cal} = a storage period calculated from regression equations based on total sensory scores.

Results and Discussion

The optimized *dudh churpi* contained (w/w) 15.4% moisture, 7.8% fat, 65.0% proteins, 4.9% total sugars and 6.9% ash (Hossain et al. 1999 c). Because of the presence of substantial amounts of proteins and sugar, the product tended to be hygroscopic. After a storage of 6 months, there was a significantly ($P<0.05$) higher adsorption of moisture in unpacked samples (i.e. kept open) compared to the packed ones (Fig. 1A). The adsorption was maximum (18.1%, w/w) in unpacked samples prepared without preservative (P_1A), and minimum (15.9%, w/w) in those prepared with sorbate and stored in glass bottles (P_2B).

An increase of 0.05% titratable acidity was observed after 6 months in the samples with sorbate and stored in glass bottles (P_2B) over the control (samples without sorbate and unpacked) (Fig. 1B). Rate of increase in the acidity was much higher, when the samples were without sorbate (P_2A). This increase might be due to microbial action or could be attributed to various chemical reactions taking place during storage, leading to the production of organic acids (Parry 1974). Potassium sorbate is known to check the development of titratable acidity during storage of *khoa* and *kalakand* (Jha et al. 1977; Suresh and Jha 1994). Initial FFA content of the product was 0.95% (w/w), which increased after 6 months of storage (Fig. 1C). Changes in the FFA profiles were less in the samples with sorbate, indicating that the release of FFA was controlled appreciably by this preservative. Samples with sorbate stored in glass bottles (P_2B) showed the least (0.04%) change in FFA content after 6 months. In *khoa*, a consistent increase in FFA content was reported during storage period irrespective of the type of pouches used for packaging. An increase in the FFA content during storage was attributed to heat resistant lipases (Law 1979). Development of FFA in *khoa* was arrested by the addition of sorbate (Jha et al. 1977; Rao et al. 1977).

Addition of sorbate adversely affected the rate of increase in tyrosine value of the samples during storage (Fig. 1D). Sorbate is effective in inhibiting the breakdown of proteins. The change in tyrosine value, which is an indication of proteolysis during storage of the samples, is believed to be due to the survival of native as well as bacterial proteases, which are not destroyed even by UHT processing (Lindquist 1970).

Both free and total HMF of *dudh churpi* were determined to evaluate the progression of Maillard browning during storage of the product (Figs. 1E and 1F). In all the cases, HMF contents increased as the storage period progressed, but the rate of increase was considerably less in the sorbate-added samples.

The least increase in HMF contents was observed in the samples with added sorbate and packed in glass bottles (P_2B). With the development of Maillard browning, there was a gradual drop in percent reflectance. In concurrence with the observation of browning, the change in reflectance was also minimum in the samples with added sorbate and stored in glass bottles, followed by plastic bottles, polyethylene pouches and unpacked ones (Fig. 1G). Regression equations for the values of moisture, HMF and reflectance showed that the changes in all the physico-chemical parameters with storage period could be explained well by the second degree equations with the correlation coefficients ranging from 0.94 to 0.99.

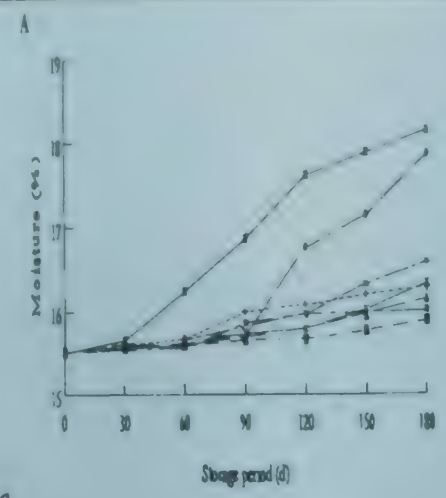
Average sensory scores of *dudh churpi*, stored under different conditions of packaging are presented in Table 1. While all the sensory attributes of control samples (P_1A) decreased very rapidly with the increase in storage period, there was minimum change of the same in the samples with sorbate and stored in glass bottles. Decrease in sensory scores with the increase in storage period could be explained as a result of interactions of all the physico-chemical parameters discussed above. Statistical analysis of the data (Table 2) revealed that three types of packages and the control samples (without packages), two types of *dudh churpi* (with or without sorbate) and the duration of storage, individually all had a significant ($P<0.01$) influence on the sensory data of *dudh*

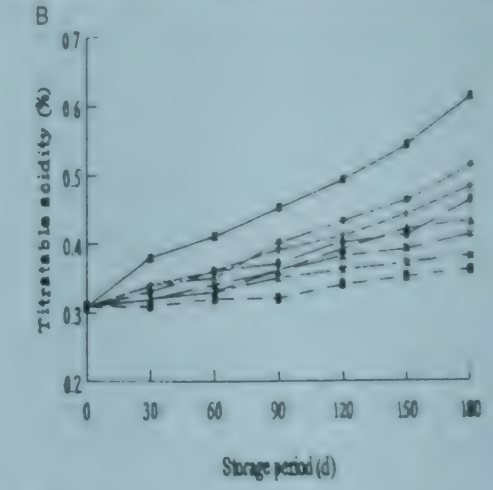
TABLE 1. SENSORY QUALITY OF *DUDH CHURPI* AS INFLENCED BY TYPES OF PACKAGINGS, STORAGE PERIODS AND A PRESERVATIVE

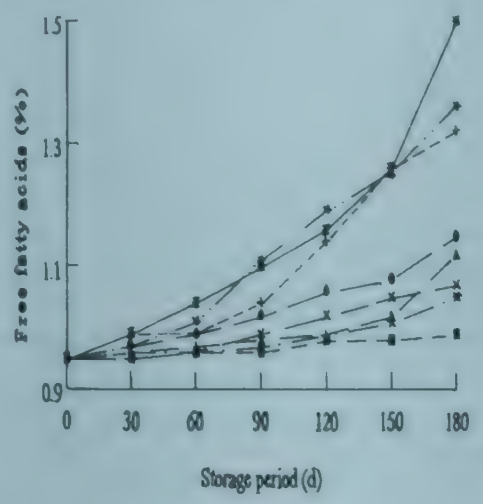
Packaging conditions[a]	Mean sensory scores of samples stored for 6 months[b]			
	Flavour	Body and texture	Colour and appearance	Total score
P_1A	3.50	5.97	1.47	10.93
P_1B	5.61	7.04	3.50	16.14
P_2A	7.79	7.60	4.11	19.57
P_2B	8.04	7.93	4.25	20.21
P_3A	7.07	7.18	3.79	18.03
P_3B	7.75	7.68	4.11	19.53
P_4A	6.79	6.64	3.36	16.78
P_4B	7.68	7.25	3.86	18.79

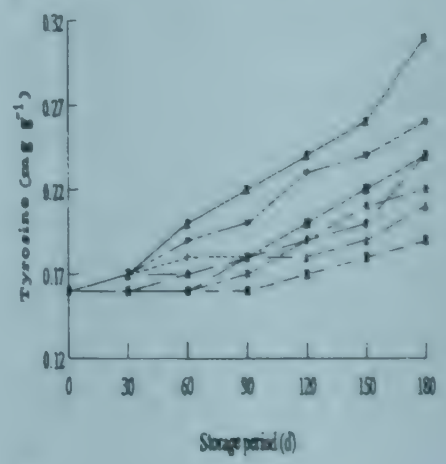
[a] P_1, unpacked; P_2, glass bottle; P_3, plastic bottle; P_4, polyethylene pouch; A, without sorbate; B, with 0.1% (w/w) potassium sorbate

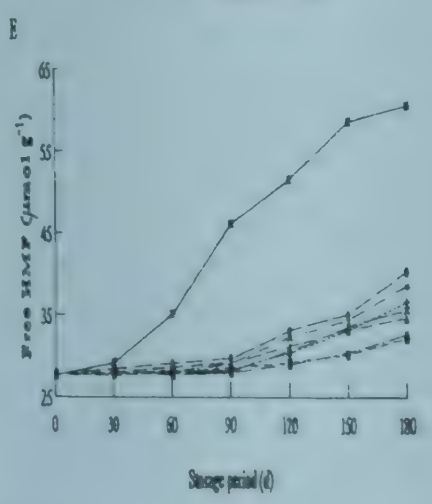
[b] Means of four replicates. Intial score (out of): flavour, 8.46 (10); body and texture, 8.36 (10); colour and appearance, 4.79 (5); total score, 21.61 (25)

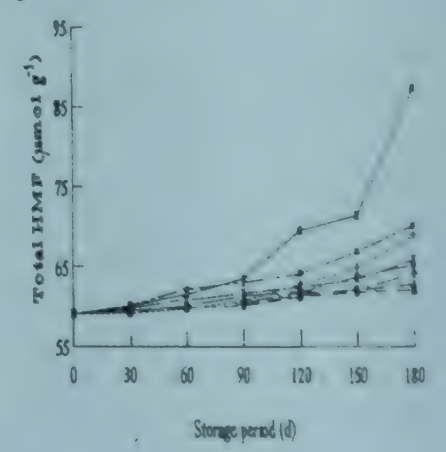


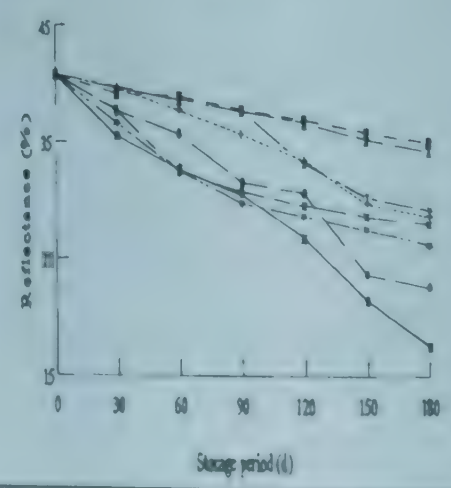












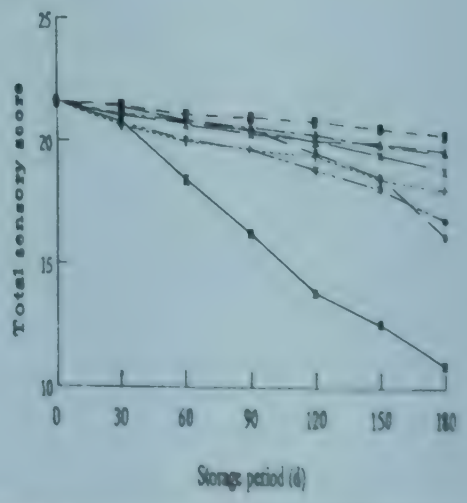


Fig. 1. Effect of packaging and storage on moisture (A), titratable acidity (B), FFA (C), tyrosine (D), free HMF (E), total HMF (F), reflectance (G) and total sensory score (H) of *dudh churpi* : P_1A (✹) without packaging and sorbate; P_1B (●), without packaging and with sorbate P_2A (▲), glass bottle and without sorbate; P_2B (■), glass bottle and with sorbate; P_3A (✚), plastic bottle and without sorbate; P_3B (•), plastic bottle and with sorbate; P_4A (★), polythylene pouch and without sorbate; P_4B (✖), polyethylene pouch and with sorbate

TABLE 2. ANALYSIS OF VARIANCE FOR SENSORY ATTRIBUTES OF *DUDH CHURPI* DURING STORAGE

Source of variation	Degrees of freedom	Flavour		Body and texture		Colour and appearance		Total scores	
		MSS	CD	MSS	CD	MSS	CD	MSS	CD
Replicates	3	0.01	-	0.03	-	0.01	-	0.01	-
Among packages (P)	3	17.24[b]	0.06	3.14[b]	0.06	7.19[b]	0.04	71.51[b]	0.10
Among storage periods (S)	6	10.81[b]	0.08	5.49[b]	0.08	6.94[b]	0.05	67.89[b]	0.13
Among preservatives (D)	1	18.42[b]	0.04	8.29[b]	0.04	10.97[b]	0.03	109.90[b]	0.07
Interactions:									
P×S	18	2.41[b]	0.16	0.46[b]	0.15	0.74[b]	0.11	8.98[b]	0.27
P×D	3	5.65[b]	0.08	0.65[b]	0.08	3.62[b]	0.06	25.63[b]	0.14
S×D	6	1.21[b]	0.11	0.53[b]	0.11	0.74[b]	0.08	6.97[b]	0.19
P×S×D	18	0.46[b]	0.22	0.12[b]	0.21	0.28[b]	0.15	2.17[b]	0.38
Error	165	0.015	-	0.014	-	0.007	-	0.043	-

[a] MSS, mean sum of squares; CD, critical difference; -, no data

[b] Significant at $P<0.01$

TABLE 3. SHELF LIFE PREDICTION OF *DUDH CHURPI* PACKED DIFFERENTLY, FOLLOWED BY STORAGE UNDER AMBIENT CONDITIONS

Packaging conditions[a]	Regression equation[b]	Coefficient of correlation (R)	RMS, %	Predicted shelf life[c] (d)
P_1A	$y = 22.05-6.31\times10^{-2}t$	- 0.99	2.402	112
P_1B	$y = 22.29-2.79\times10^{-2}t$	- 0.93	3.357	261
P_2A	$y = 21.44-1.09\times10^{-2}t$	- 0.96	0.487	592
P_2B	$y = 21.63-0.76\times10^{-2}t$	- 0.93	0.286	871
P_3A	$y = 21.63-1.88\times10^{-2}t$	- 0.97	0.918	339
P_3B	$y = 21.53-1.13\times10^{-2}t$	- 0.97	0.302	577
P_4A	$y = 21.63-2.50\times10^{-2}t$	- 0.98	1.134	265
P_4B	$y = 21.59-1.48\times10^{-2}t$	- 0.96	0.493	444

[a] P_1, without packaging; P_2, glass bottle; P_3, plastic bottle; P_4, polyethylene pouch; A, without sorbate; B, with 0.1% (w/w) potassium sorbate

[b] y, overall acceptability (total score); t, period of storage (d)

[c] Calculated on the basis of minimum acceptability score of 15

churpi. Interaction between packages and storage period, packages and preservatives, storage period and preservative and storage period, preservative and packages, were significant ($P<0.01$), indicating that storage period, package and preservatives jointly influenced the sensory quality of *dudh churpi* during storage. Thus, the application of additional hurdles i.e. potassium sorbate and packaging are effective in extending the shelf stability of the product (Leistner 1992).

Moisture adsorption, oxidation and lipolysis were found to take place in the product during storage. The changes in multiplicity of physico-chemical parameters like contents of moisture, HMF, FFA and reflectance governed the sensory scores of the product. The regression equations of physico-chemical parameters and total sensory score explained the product's quality during storage up to the point of acceptability.

Shelf life of the product was assessed from the regression line of the mean overall acceptability scores or total scores on storage period, assuming the total score of 15 to be the limit, below which the product was not acceptable. The relationships of overall acceptability and the shelf life of the product are shown in Fig. 1H and Table 3. The low % RMS (0.286 to 3.357) showed that the equations fitted well to the data. The predicted shelf life of *dudh churpi* with added sorbate and stored in glass bottles was 871 days, which was 4-fold higher than those of control samples and 8-fold higher than the samples without sorbate and stored traditionally.

Thus, the shelf life of *dudh churpi* under ambient condition of storage was extended with the incorporation of 0.1% (w/v) potassium sorbate in milk used for cooking pre*churpi* and by packaging. The shelf life was maximum, when the product was stored in glass bottles.

The mathematical model developed to predict shelf life of *dudh churpi* should find potential applications in prospective commercial establishments from where large batches of products will be lifted for marketing in diverse marketing destinations.

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RESEARCH PAPER

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Isolation, Biochemical Characterization and Antibiotic Susceptibility of *Yersinia enterocolitica* Isolates from Milk

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Yersinia enterocolitica isolates of milk origin were biochemically characterized using presumptive and primary identification schemes. Additional biochemical tests were carried out to confirm the typical isolates. On the basis of colony morphology on Cefsulodin-Irgasan-Novobiocin (CIN) agar, 65 samples out of 110 were found to have typical *Yersinia* colonies. The alkali (KOH+ NaCl) treatment increased the selectivity of the isolation procedure. On the basis of H_2S negative, urease positive and esculin negative tests, 58 isolates were identified as presumptive *Yersinia*. These were screened for antibiotic susceptibility by disc assay. The results revealed that gentamycin (10 µg) and streptomycin (10 µg) were the most effective. On the other hand, penicillin G (10 units) was most ineffective, as about 54 culture isolates were completely resistant to this antibiotic. Further tests for oxidase negative, catalase positive and lipase negative or delayed positive resulted in the primary identification of 47 isolates on *Yersinia enterocolitica*. The additional biochemical tests for confirmation of the typical strains were negative decarboxylase test for lysine and arginine, inability to deaminate phenylalanine, motility at 25°C, a negative response to citrate utilization and VP test and negative or delayed positive indole production. A total of 36 isolates were identified as confirmed *Yersinia enterocolitica*. A number of sugar fermentation tests were also conducted to substantiate the results.

Keywords : Antibiotic susceptibility, *Yersinia enterocolitica*, Isolation, Characterization, Milk.

Yersinia enterocolitica is an emerging pathogen of importance of milk-borne diseases. It is one of the few human pathogens that can grow at refrigeration temperatures and its presence in milk is of great public health significance. The animal kingdom is a significant reservoir of *Y. enterocolitica* (Swaminathan et al. 1982). The organism has also been isolated from dairy cattle (Esselveld and Gouzaard 1973; Inoue and Kurose 1975; Wooley et al. 1980) and has also been reported to be indigenous to the gastrointestinal tract of the animals. Since the organism is known to be transmitted through milk, information about its antibiotic susceptibility is of great significance. Some studies have been carried out in the past that revealed the variable antibiotic susceptibility pattern of *Yersinia* isolates (Delmas and Vidon 1982; Tibana et al. 1987; El-Kholy 1992).

It is increasingly becoming important to standardize cultural methods for its isolation and biochemical tests for its identification. The organism is unique among enteric pathogens in its ability to grow at 4°C and this property has been utilized by several investigators for selective enrichment from foods and other sources (Otsuki et al. 1973; Tsuobokura et al. 1976; Pederson 1976; Hanna et al. 1976). Cold enrichment techniques using phosphate buffered saline (PBS) at 4°C for 4 weeks tried by several workers (Chen et al. 1988; VanPee and Stragier 1979; Weissdeld and Sonnenwirth 1980) were found to be unsuitable primarily because of the long incubation period. An addition of 1% sorbitol and 0.15 % bile salts No. 3 to PBS was shown to enhance recovery of *Y. enteroco-litica* (Mehlman et al. 1978). The use of Peptone Sorbitol Bile (PBS) broth was also recommended as a standard method for the enrichment of the organism (Marshall 1992). At the same time, Aulisio et al (1980) observed that *Y. enterocolitica* tolerated short exposure to weak alkali better than other members of the family enterobacteriaceae. An exposure to 0.5% KOH and 0.5% NaCl was reported to increase the yield of organism four-fold and the sensitivity 100-fold. The alkali treatment was also reported to decrease the growth of compet-ing bacteria. In a later study, Franzin et al (1984) reported a cold enrichment method with phosphate buffered saline and sorbitol-bile-salts followed by treatment with KOH in NaCl and plating on selective agar media, increased the isolation of *Y. enterocolitica* from raw milk.

Strains of the organism showed a remarkable degree of variability in their ability to grow on selective media, commonly used for the isolation of enteric pathogens (Otsuki et al. 1973). It was reported that, on media such as Endo, Desoxycholate, MacConkey and *Salmonella Shigella* agar, the colonies developed slowly, attaining a diameter of only 0.05 mm after an incubation period of 24 h at 22 or 37°C. These small colonies, could thus be easily overlooked, particularly in the presence of large number of other enteric bacteria. In a later study, Schiemann (1979 a) formulated a new selective medium, Cefsulodin-Irgasan-Novobiocin (CIN) agar to provide quantitative recoveries of 40 strains of *Y. enterocolitica* and was reported to be inhibitory to *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis*. The efficiency of CIN agar for high recovery of all *Yersinia* spp. was also reiterated by Walker and Gilmour (1986)

Strains of *Y. enterocolitica* are biochemically heterogenous. Various schemes were proposed in the past, necessitating the importance to specify the scheme used. Primary screening of suspect colonies of *Y. enterocolitica* from selective differential plating media was accomplished by inoculating from each selected colony to a triple sugar iron (TSI) agar slant, mannitol broth and lysine decarboxylase medium. Cultures, which gave non-fermentative reactions produced H_2S, failed to ferment mannitol or decarboxylate lysine were rejected (Mehlman et al. 1978). After a suspect culture satisfied the criteria of primary screening, it was subjected to secondary

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and tertiary stages of biochemical tests to confirm the presence of *Y. enterocolitica*.

In view of the variability in biochemical characterisation and lack of data on the identification schemes and antibiotic susceptibility pattern of *Yersinia*, the present investigation was undertaken to isolate, biochemically characterize and study the antibiotic susceptibility of *Y. enterocolitica* isolates of milk origin.

Materials and Methods

A total of 110 milk samples wee collected from different sources, mainly originating from the villages of Haryana. These constituted 80 raw milk and 30 pasteurized milk samples. The aseptically drawn milk samples were analysed immediately for *Y. enterocolitica* and were characterized biochemically using standard procedures (Marshall 1992).

Isolation : For the isolation of *Yersinia*, a 25 ml of well mixed milk sample was aseptically transferred to 225 ml peptone-sorbitol-bile (PSB) broth. The thoroughly mixed sample was incubated at 10°C for 10 days in a BOD incubator.

After incubation, the enriched samples were mixed thoroughly and 0.1 ml of each was transferred to 1 ml of sterilized 0.5% KOH solution in 0.5% saline and vortexed for 5 sec.

The alkali-treated samples were immediately streaked on to pre-poured Cefsulodin-Irgasan-Novobiocin (CIN) agar plates. The plates were incubated at 25°C for 48 h and were examined for small (1-2 mm in diameter) colonies having a deep red centre (Bull's eye) with sharp borders surrounded by a clear colourless zone with an entire edge.

Identification and characterization : The selected colonies from CIN agar plates were identified using standard biochemical tests (Marshall 1992).

Presumptive testing : A representative number of typical *Yersinia* colonies was picked up from the CIN agar plates and transferred to BHI broth. After 24 h of incubation, the cultures were tested for H_2S production, on lysine iron agar (LIA) slants, esculin hydrolysis and urease production.

Primary identification : The presumptive positive isolates were further tested by oxidase, catalase and lipase tests in addition to Gram staining.

Confirmation : Several additional biochemical tests were carried out to confirm the *Y. enterocolitica* isolates. A distinctive feature of *Yersinia* is the temperature dependence of a number of biochemical reactions. However, in general, an incubation temperature of 25°C is recommended for biochemical reaction (Varnam and Evans 1991).

Different tests used for confirmed identification were decraboxylation of lysine, arginine and ornithine, deamination of phenylalanine, motility at 25° and 37°C and citrate utilization. In addition to this, the sugar fermentation tests were carried out by inoculating the isolates in Bromo Cresol Purple (BCP) broth supplemented individually with the following carbohydrates, each at 0.5 % level: mannitol, sorbitol, sucrose, trehalose, salicin and D-xylose.

TABLE 1. COLONY MORPHOLOGY AND CHARACTERISTICS OF *YERSINIA* ISOLATES FROM MILK ON CIN AGAR AT 25°C

Sample numbers	Plating on CIN Agar NaCl	KOH+NaCl (Treated)	Typical Bull's eye colony	Margin	Diameter, mm
6, 18, 72, 73, 78 86, 90-93, 95-98	+	+	+	S	<1.0
1, 2, 4, 5, 8, 12, 13, 15, 20, 21, 22, 24, 26, 28, 30, 41, 43, 47, 49, 71, 74, 75, 76, 77, 79, 80, 82, 83, 84, 87, 88, 89, 94, 99, 100-102, 105-110	+	+	+	S	1-1.15
9-11, 27, 81, 103, 104	+	+	+	S	1.6-2
31-40, 51-70	-	-	-	-	-
3, 7, 14, 16, 17, 19, 23, 25, 29, 42, 44-46, 48, 50	+	-	-	-	-

- = no colonies observed, S= smooth margin

TABLE 2. PRESUMPTIVE CHARACTERIZATION OF *YERSINIA* ISOLATES

S. Nos.	LIA H_2S	LIA Alk	LIA Acid	LIA Gas	Urease at 37°C	Esculin at 37°C	Presumptive *Yersinia*
1-4, 7,8, 10-32, 34-39, 41-45, 47-49, 51-65	-	+	+	-	+	-	+
5, 6, 9, 33, 40, 46, 50	-	+	-	-	-	-	-

TABLE 3. PRIMARY CHARACTERIZATION OF *YERSINIA ENTEROCOLITICA* ISOLATES

S. Nos.	Lipase	Catalase	Oxidase	Gram reaction	Primary Identification
1, 2, 4, 5, 8, 9, 11, 13, 15, 16, 18, 19-24, 28-38, 41, 43-47, 49-52 54-57	-	+	-	-	+
3, 6, 7, 10, 14, 17, 26, 40, 42 48, 53	+	+	(+)	-	-
12, 25, 27, 39, 58	+	+	-	-	+

(+) = delayed positive

TABLE 4. ADDITIONAL TESTS FOR BIOCHEMICAL CHARACTERIZATION OF *YERSINIA ETEROCOLITICA* ISOLATES AT 25°C

S. No.	Decarboxylase test			Deamination of	Motility test		Indole	VP test		Citrate	Confirmed
	Lysine	Arginine	Ornithine	phenylalanine	25°C	37°C	prodn.	25°C	37°C	utilization	isolates
1, 2, 4, 13, 15, 16, 19, 35, 45-47, 50, 51, 54, 55	-	-	-	-	+	-	-	+	-	-	+
9	-	-	-	-	+	-	(+)	+	-	-	+
12, 18, 21-24, 31-34, 36, 39 41, 43, 44, 57	-	-	+	-	+	-	-	+	-	-	+
25, 27, 58	-	-	+	-	+	-	(+)	+	-	-	+
52	-	-	+	-	+	-	-	+	-	-	+
3, 7, 26, 42, 48, 53	-	-	-	+	+	-	-	+	-	-	+
28, 29, 30	-	-	-	+	+	-	(+)	-	-	+	-
5, 11, 20 37, 38, 56		-	-	-	+	-	+	+	-	+	-
49	-	-	-	-	+	-	+	+	-	+	-
6, 40	-	-	-	+	+	-	+	-	-	+	-
8, 10	-	-	-	+	+	-	-	-	-	+	-
14, 17	-	-	+	+	+	-	-	-	-	+	-

(+) = Delayed pobisitive

TABLE 5. SUGAR FERMENTATION TESTS OF *Y. ENTEROCOLITICA* ISOLATES AT 25°C

S. Nos.	Sucrose	Trehalose	D-Xylose	Mannitol	Salicin	Sorbitol
1, 8, 17 28, 29, 37, 42, 49-51, 54-57	+	-	+	+	+	+
2, 18, 19, 21-25, 27, 31, 32, 34, 36, 39, 41, 43, 44, 46, 47, 52, 58	+	+	+	+	+	+
3, 7	-	-	-	-	-	+
4, 9	+	+	-	+	+	+
5, 26, 38	-	+	+	+	+	+
6, 10, 14	-	+	-	+	-	+
11, 13	+	-	-	+	+	+
15, 16	+	+	-	+	-	+
33	+	+	-	-	-	+
20, 30, 35 40, 48, 53	-	-	+	+	+	+

Antibiotic susceptibility : The isolates were further screened for antibiotic susceptibility at 25°C on Mueller Hinton agar by standard disc assay (Bauer et al. 1966). The set of antibacterial agents was as recommended by Washington and Barry (1974). The prepared antibacterial discs were obtained from Hi-media (India) and the results were compared by the antibiotic susceptibility chart as sensitive (S), resistant (R) and intermediate (I).

Results and Discussion

On the basis of colony morphology on CIN agar, 65 raw milk samples out of the total of 110 were found to contain typical *Yersinia* colonies (Table 1). On the other hand, none of the pasteurised milk samples was positive. Although so far, there are no reports on the incidence of *Yersinia enterocolitica* in milk in our country, several workers have reported its presence in raw and pasteurised milk worldwide with a distinct incidence in many countries (Esselveld and Guudzaard 1973; Schiemann 1979 b; Christensen, 1982; Tibana et al. 1987; Chen et al. 1988; Pasetto 1991; Rohrbach et al. 1992; Ados et al. 1994).

As regards alkali treatment of the samples, it was noticed that KOH + NaCl treatment indicated better selectivity as compared to NaCl treatment alone. The resistance of *Yersinia* to pH and the consequent decrease of background flora has been successfully exploited for isolation of the organism (Aulisio et al. 1980). Hence, the present observations are in concurrence with the above findings regarding the selectivity of alkali treatment for isolation of typical *Yersinia.*

For the presumptive characterization of the isolates, the typical *Yersinia* isolates were H_2S negative, produced alkaline reaction on LIA slant and acidic in but without gas production. In addition, these were urease positive and esculin negative (Table 2). Based on these tests, 58 isolates were identified as presumptive *Yersinia.* Similar tests have been used by other workers for preseumptive testing of *Yersinia* spp. (Mehlman and Auliso 1978; Tibana et al. 1987).

Primary characterization of *Y. enterocolitica* involved Gram-negative reaction of the rods that were oxidase negative,

TABLE 6 ANTIBIOTIC SUSCEPTIBILITY OF PRESUMPTIVE *YERSINIA* ISOLATES

Chloramphenicol	Gentamycin	Nalidixic Acid	Nitrofurantoin	Penicillin G	Streptomycin	Co-trimaxazole	Tetracyclin
1.7 (I)	2.5 (S)	1.5 (I)	NZ (R)	NZ (R)	1.6 (S)	NZ (R)	2.0 (S)
1.5 (I)	2.5 (S)	1.5 (I)	NZ (R)	NZ (R)	1.5 (S)	NZ (R)	2.0 (S)
1.5 (I)	2.5 (S)	1.5 (I)	NZ (R)	NZ (R)	2.0 (S)	1.0 (R)	2.4 (S)
2.0 (S)	2.0 (S)	2.3 (S)	NZ (R)	NZ (R)	1.7 (S)	1.0 (R)	2.6 (S)
1.6 (I)	2.7 (S)	2.2 (S)	NZ (R)	NZ (R)	1.8 (S)	NZ (R)	1.6 (S)
1.5 (I)	3.0 9S)	1.8 (I)	NZ (R)	NZ (R)	1.6 (S)	NZ (R)	2.3 (S)
1.3 (I)	2.5 (S)	1.4 (I)	NZ (R)	NZ (R)	2.0 (S)	NZ (R)	1.4 (R)
1.2 (R)	2.3 (S)	2.0 (S)	NZ (R)	NZ (R)	1.7 (S)	NZ (R)	1.2 (R)
1.5 (I)	2.5 (S)	2.0 (S)	NZ (R)	NZ (R)	1.5 (S)	NZ (R)	1.4 (R)
1.6 (I)	2.2 (S)	2.4 (S)	NZ (R)	NZ (R)	2.3 (S)	NZ (R)	2.0 (S)
1.8 (S)	2.5 (S)	2.0 (S)	NZ (R)	NZ (R)	2.0 (S)	NZ (R)	1.5 (S)
2.5 (S)	2.5 (S)	2.5 (S)	NZ (R)	NZ (R)	2.0 (S)	1.5 (I)	2.0 (S)
2.1 (S)	2.6 (S)	2.0 (S)	NZ (R)	NZ (R)	1.9 (S)	NZ (R)	2.0 (S)
3.2 (S)	2.7 (S)	3.4 (S)	2.0 (S)	NZ (R)	1.7 (S)	NZ (R)	2.5 (S)
1.6 (I)	2.6 (S)	1.6 (I)	NZ (R)	NZ (R)	2.8 (S)	NZ (R)	2.2 (S)
1.7 (I)	2.6 (S)	1.9 (S)	NZ (R)	NZ (R)	2.0 (S)	0.7 (R)	2.0 (S)
2.2 (S)	2.5 (S)	1.7 (I)	NZ (R)	1.0 (R)	2.0 (S)	1.5 (I)	2.2 (S)
2.8 (S)	2.5 (S)	3.0 (S)	1.5 (I)	NZ (R)	2.2 (S)	2.8 (S)	1.5 (I)
1.6 (I)	2.5 (S)	1.6 (I)	1.7 (S)	NZ (R)	1.6 (S)	NZ (R)	2.0 (S)
2.4 (S)	2.5 (S)	2.0 (S)	1.7 (S)	NZ (R)	2.1 (S)	1.5 (1)	2.0 (S)
3.0 (S)	2.5 (S)	3.2 (S)	1.5 (I)	NZ (R)	1.6 (S)	NZ (R)	2.0 (S)
2.5 (S)	2.4 (S)	2.6 (S)	1.5 (I)	1.0 (R)	2.5 (S)	1.5 (1)	2.5 (S)
2.3 (S)	2.3 (S)	2.4 (S)	1.7 (S)	NZ (R)	2.0 (S)	NZ (R)	2.5 (S)
2.5 (S)	2.0 (S)	3.0 (S)	1.5 (I)	NZ (R)	2.8 (S)	2.5 (S)	2.4 (S)
2.8 (S)	2.5 (S)	2.6 (S)	1.7 (S)	NZ (R)	2.2 (S)	3.2 (S)	2.5 (S)
2.5 (S)	2.5 (S)	3.0 (S)	1.5 (I)	NZ (R)	2.4 (S)	NZ (R)	2.8 (S)
2.0 (S)	2.4 (S)	2.5 (S)	NZ (R)	NZ (R)	2.5 (S)	NZ (R)	2.6 (S)
2.0 (S)	2.4 (S)	2.4 (S)	NZ (R)	NZ (R)	2.0 (S)	2.2 (S)	2.4 (S)
1.8 (S)	2.0 (S)	2.5 (S)	NZ (R)	NZ (R)	1.6 (S)	NZ (R)	2.6 (S)
2.5 (S)	2.4 (S)	3.0 (S)	NZ (R)	NZ (R)	1.5 (S)	NZ (R)	3.0 (S)
3.0 (S)	2.0 (S)	2.0 (S)	NZ (R)	NZ (R)	2.2 (S)	NZ (R)	2.4 (S)
3.2 (S)	2.5 (S)	2.6 (S)	1.5 (I)	NZ (R)	2.5 (S)	2.5 (S)	2.5 (S)
2.0 (S)	2.3 (S)	2.0 (S)	NZ (R)	NZ (R)	2.0 (S)	NZ (R0	2.0 (S)
1.8 (S)	2.4 (S)	3.2 (S)	NZ (R)	NZ (R)	2.5 (S)	3.0 (S)	2.8 (S)
3.0 (S)	2.0 (S)	2.0 (S)	1.5 (I)	NZ (R)	2.3 (S)	3.0 (S)	2.4 (S)
2.5 (S)	2.2 (S)	2.5 (S)	1.4 (R)	NZ (R)	2.0 (S)	2.5 (S)	2.8 (S)
2.5 (S)	2.5 (S)	2.5 (S)	2.0 (S)	NZ (R)	2.5 (S)	NZ (R)	3.0 (S)
2.4 (S)	2.2 (S)	3.0 (S)	1.6 (I)	NZ (R)	2.2 (S)	3.0 (S)	2.0 (S)
1.6 (S)	2.6 (S)	2.0 (S)	1.5 (I)	NZ (R)	2.2 (S)	2.0 (S)	2.4 (S)
2.0 (S)	2.5 (S)	2.7 (S)	1.7 (S)	1.5 (S)	2.3 (S)	1.5 (I)	2.5 (S)
2.5 (S)	2.4 (S)	3.2 (S)	1.5 (I)	NZ (R)	2.0 (S)	3.3 (S)	2.4 (S)
2.5 (S)	2.5 (S)	3.0 (S)	1.5 (I)	1.5 (S)	2.5 (S)	2.4 (S)	2.5 (S)
2.7 (S)	2.5 (S)	3.0 (S)	1.5 (I)	1.6 (S)	2.2 (S)	3.0 (S)	2.5 (S)
3.0 (S)	2.5 (S)	3.2 (S)	1.5 (I)	1.2 (R)	3.0 (S)	2.5 (S)	2.5 (S)
3.0 (S)	2.3 (S)	3.0 (S)	1.7 (S)	1.7 (S)	2.0 (S)	3.0 (S)	2.7 (S)
1.5 (I)	2.3 (S)	2.0 (S)	1.7 (S)	1.0 (R)	2.0 (S)	1.0 (R)	2.5 (S)
3.0 (S)	2.0 (S)	2.0 (S)	1.5 (I)	NZ (R)	1.5 (S)	NZ (R)	2.0 (S)
3.0 (S)	2.0 (S)	2.0 (S)	1.5 (I)	NZ (R)	1.5 (S)	NZ (R)	2.0 (S)
2.5 (S)	2.5 (S)	2.0 (S)	2.0 (S)	NZ (R)	1.5 (S)	1.2 (I)	2.5 (S)
2.0 (S)	2.0 (S)	2.0 (S)	1.7 (S)	NZ (R)	2.0 (S)	2.5 (S)	2.0 (S)
3.0 (S)	2.0 (S)	2.0 (S)	NZ (R)	NZ (R)	2.2 (S)	NZ (R)	2.4 (S)
1.6 (I)	2.5 (S)	2.5 (S)	1.5 (I)	NZ (R)	1.5 (S)	NZ (R)	1.5 (I)
1.8 9S)	2.5 (S)	1.8 (I)	1.5 (I)	1.0 (R)	1.5 (S)	1.3 (I)	2.4 (S)
2.5 (S)	2.0 (S)	2.7 (S)	1.7 (S)	NZ (R)	2.0 (S)	2.5 (S)	2.4 (S)
3.0 (S)	2.0 (S)	2.5 (S)	2.0 (S)	NZ (R)	2.3 (S)	3.0 (S)	2.5 (S)
1.2 (R)	2.0 (S)	1.4 (I)	1.5 (I)	NZ (R)	1.5 (S)	NZ (R)	2.0 (S)
3.0 (S)	2.0 (S)	3.0 (S)	2.0 (S)	NZ (R)	2.5 (S)	1.5 (I)	2.4 (S)
2.5 (S)	1.7 (S)	2.0 (S)	1.5 (I)	1.0 (R)	2.0 (S)	2.5 (S)	2.0 (S)

catalase positive and negative or delayed positive for lipase (Bercovier and Mollaret 1984). On the basis of these tests, 47 isolates were primarily identified as *Y. enterocolitica* (Table 3). The results presented also reflect variation in the characteristics of the isolates with regard to the tests. The oxidase test, along with Gram-reaction and catalase test was previously also used for identification of *Y. enterocolitica* at a primary level (Marshall 1992).

A number of additional biochemical tests were carried out for confirmation of *Y. enterocolitica*. On the basis of negative decarboxylase test for lysine and arginine, inability to deaminate phenylalanine, motility at 25°C, a negative response to citrate utilization and Voges Proskauer test (at 37°C)and negative on delayed positive for indole production, 36 out of 47 primary identified isolates were confirmed as *Y. enterocolitica* (Table 4). The tests that showed variable results were decarboxylation of ornithine and VP test at 25°C. Different fermentation tests were also included in the confirmatory biochemical characterization scheme of the organism. The specific tests that proved useful in identification of isolates were acid from sucrose, xylose and trehalose. All 36 confirmed *Y. enterocolitica* isolates were also positive for the three sugars (Table 5). The additional tests were fermentation of mannitol, sorbitol and salicin, that showed variable reactions for the isolates.

The results presented in Table 6 depict the susceptibility pattern of the isolates against different antibiotics. It may be observed that gentamycin (10 µg) and streptomycin (10 µg) were the most effective as 100 % of the isolates were completely sensitive to these antibiotics. Tetracyclin (30 µg) was effective against 52 isolates and only 3 isolates were resistant to this antibiotic. Nalidixic acid (30 µg) and chloramphenicol (30 µg) were effective against 48 and 41 isolates, respectively. Although 10 isolates showed intermediate sensitivity to nalidixic acid, none of the isolates was resistant. On the other hand, with 15 intermediate isolates, at least 2 isolates were completely resistant to chloramphenicol.

The antibiotic penicillin G (10 units) was most ineffective against the isolates, as about 54 culture isolates were completely resistant to this antibiotic. Production of β-lactamase by many strains of *Yersinia* was reported to be one of the reasons for ineffectiveness of penicillin and cephalosporin (Botton 1977). Similarly, co-trimoxazole (25 µg) also revealed a selective inhibition pattern with 19 sensitive cultures, 10 intermediate cultures and 29 resistant cultures. The present study, thus, suggests a general variation in the sensitivity pattern of the isolates obtained from milk from different sources. As such, no regional patterns could be established in this case.

The antibiotic susceptibility is of great importance in resistance monitoring of the foodborne pathogens for their effective control. Many of the previous studies have also indicated a great variation in the susceptibility pattern of the *Yersinia* isolates (El-Kholy 1992; Franzin et al. 1984; Tibana et al. 1987). Hence, the present findings are in concurrence with these reports and also form the report on the susceptibility pattern of *Y. enterocolitica* isolates of milk origin in this country.

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RESEARCH NOTE

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Studies on Ripening Changes in Mango (*Mangifera indica* L.) Fruits

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Studies were carried out to determine the qualitative and quantitative changes in physico-chemical characteristics of mango fruits during ripening by making use of eight mango hybrids or varieties. The major changes were reduction in fruit weight (351 to 306 g), volume (338 to 309 ml), fruit length (11.04 to 10.55 cm), thickness (6.91 to 6.51 cm), firmness (28.96 to 17.46 lbs/sq.inch), pulp content (77.53 to 75.14%), pulp to peel ratio (7.83 to 6.23), acidity (2.71 to 0.04%), starch (10.7 to 0.43%), vitamin- C (40.83 to 11.08 mg/100 g) and increase in peel (9.00 to 12.06%), TSS 8.55 to 19.00° Brix) pH (2.85 to 4.38), total sugars (2.69 to 11.16%), sugar:acid ratio (0.99 to 27.22) and carotenoids (498 to 8071 µg/ 100g). The peel colour turned from light green or green or dark green to light yellow or yellow or orange yellow, whereas pulp colour changed from white or pale yellow to yellow or deep yellow or orange yellow.

Keywords : Mango fruits, Unripe, Ripe, Changes, Physical composition, Chemical constituents.

Mango (*Mangifera indica* L) fruit is utilised at all stages of its development, from immature stage to ripe condition. Since mango is a climacteric fruit, normal ripening takes place off the tree. Therefore, the fully mature mango fruits are harvested and stored under hay or suitable storage conditions for proper ripening either for fresh marketing or for processing. The physiology of ripening involves numerous activities, resulting in loss of fruit weight and volume, changes in specific gravity, peel and pulp colour, decline in fruit firmness followed by a series of changes in chemical composition of pulp such as decline in acidity, starch and alcohol insoluble solids and increase in total soluble solids, sugars, carotenoids and aroma forming compounds. Though, many workers have reported changes in physico-chemical characteristics of different mango varieties or hybrids, no information is available about the extent of changes in these parameters in mango fruit as such. Therefore, the present studies were carried out to find out the quantitative changes in physical characteristics, physical composition and chemical constituents of mango fruits, during ripening, by making use of eight different mango hybrids or varieties.

Raw materials : Fully mature fruits of commercial cultivar 'Alphonso' and seven hybrids viz., 'Arka Aruna', 'Arka Puneet', 'Arka Anmol', 'Arka Neelkiran' (all developed at IIHR, Bangalore) 'Mallika' and 'Amrapali' (Developed at IARI, New Delhi) and 'Ratna' (Developed at Konkan Krishi Vidyapeet, Dapoli) were used in this study. Freshly harvested fruits either from IIHR Farm or University of Agricultural Sciences, Bangalore were brought to the laboratory, sorted out for uniform maturity by putting in plain water, washed, soaked in 0.5% bavistin fungicide for 5 min and surface-dried. The selected 5.00 kg fruits of each hybrid or variety replicated four times were stored in cardboard boxes at room temperature (18-34°C) and allowed to ripen to optimum condition without any other treatments.

Physical characteristics of fruits : At the time of storage and also at the end of storage period, 10 fruits were selected randomly from each lot and physical parameters of fruits such as weight, volume, specific gravity, length, breadth and thickness were recorded using an electronic balance, water displacement method, formula and vernier calipers (Mitutoya,. Japan), respectively. The fruit firmness was determined using a penetrometer (FT 327, Effegi, Italy). The visual colours of peel and pulp were also recorded in unripe and ripe fruits. The fruits were further washed, hand-peeled and pulp was removed completely from peel as well as stone with the help of a stainless steel knife. The weights of different fruit components such as peel, pulp and stone were recorded using an electronic balance and expressed in percentage. The differences in physical parameters of same unripe and ripe fruits were obtained by deduction and percent change was worked out based on the unripe fruit values.

Chemical composition of fruits : The pulp obtained from fully ripe fruits and edible part of unripe fruits (at the time of storage) of different fruits was blended in a Waring blender and used for the determination of chemical constituents of unripe and ripe fruits. The total soluble solids (TSS) of the pulp were determined by a hand refractometer (Erma, 0-32°Brix, Japan) at room temperature and necessary temperature corrections were applied. Acidity, reducing and total sugars, vitamin C and carotenoids of pulp were determined by adopting the methods given by Ranganna (1991). The starch content was determined by the method of Stewart et al (1974). The pH of the pulp was determined by a digital pH meter (Elico, Hyderabad), while the viscosity of the pulp was determined by a Viscometer (LVTD, Brookfield, USA). The differences in chemical constituents of unripe and ripe fruits were obtained by deduction and per cent changes were worked out based on unripe fruit values. All the data were subjected to statistical analysis using factorial CRD design (Sundararaj et al. 1972) and F-test was applied at 5% level.

Physical characteristics of fruits : The data pertaining to the physical characteristics of mango fruit, as influenced by

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TABLE 1 CHANGES IN PHYSICAL CHARACTERISTICS OF MANGO FRUITS DUE TO RIPENING

Condition of fruit	Weight, g	Volume, ml	Specific gravity	Length, cm	Breadth, cm	Thickness, cm	Firmness, lbs/sq.inch
Unripe	351	338	1.04	11.04	7.87	6.91	28.96
Ripe	306	309	0.99	10.55	7.70	6.51	17.46
Change	- 45	- 29	- 0.05	- 0.49	- 0.17	- 0.40	- 11.50
Per cent change	12.8	- 8.6	- 4.81	- 4.43	- 2.16	- 5.78	- 39.71
SEm ±	7.0	7.0	0.02	0.13	0.13	0.05	0.29
CD at 5%	21.0	19.0	NS	0.38	NS	0.15	0.83

TABLE 2. CHANGES IN PHYSICAL COMPOSITION AND COLOUR OF MANGO FRUITS DUE TO RIPENING

Condition of fruit	Peel, %	Pulp, %	Stone, %	Pulp:Peel, ratio	Colour of fruits	Colour of pulp
Unripe	9.90	77.53	12.57	7.83	Light green or green to dark green	White to pale yellow
Ripe	12.06	75.14	12.80	6.23	Light yellow or yellow to orange yellow	Yellow or deep yellow to orange yellow
Change	+ 2.16	- 2.39	0.23	1.60		
Per cent change	+ 21.82	- 3.08	1.83	20.42	-	-
SEm ±	0.15	0.26	0.26	0.10	-	-
CD at 5%	0.42	0.76	NS	0.29	-	-

ripening are presented in Table 1. Ripening resulted in a significant losses of fruit weight and volume. The loss in weight (12.8%) was more than the volume (8.6%). The reduction in fruit weight was attributed to the physiological loss in weight (PLW) due to transpiration of water through peel tissue. This physiological loss in weight is influenced by size of a fruit, storage temperature (Srivastava 1967) and variety (Ram and Date 1971; Lakshminarayan 1973, Chikkasubbanna and Huddar 1982). The loss in fruit volume was less than weight since the reduction in size due to PLW was not proportional to the weight of water lost. Ripening reduced the fruit length by 4.43% and thickness by 5.78%, but reduction in fruit breadth was not significant. The reductions in length and thickness of fruits were attributed to shrivelling of fruits due to loss of water from fruits. Among all the physical characteristics studied, firmness (Fig. 1) was affected much more (39.71%) than any other parameter. This reduction could be attributed to the breakdown of insoluble pectin substances to soluble forms and these pectin polymers became less tightly bound in the cell walls during ripening. According to Tandon and Kalra (1984), the declining concentration of calcium might reduce calcium pectin interaction, allowing free release into the flesh leading to reduced firmness as the fruits ripen, resulting in shrivelling of over-ripe mango fruits.

Physical composition and colour of fruits : Statistically significant differences were observed in the physical composition and colour of mango fruits due to ripening (Table 2). Ripening resulted in increase of peel content, whereas pulp content reduced. Loss of water from the pulpy portion and breakdown of certain complex substances to simple forms during ripening was the main cause of reduction in the pulp weight, leading to lowering of pulp yield and increase of peel

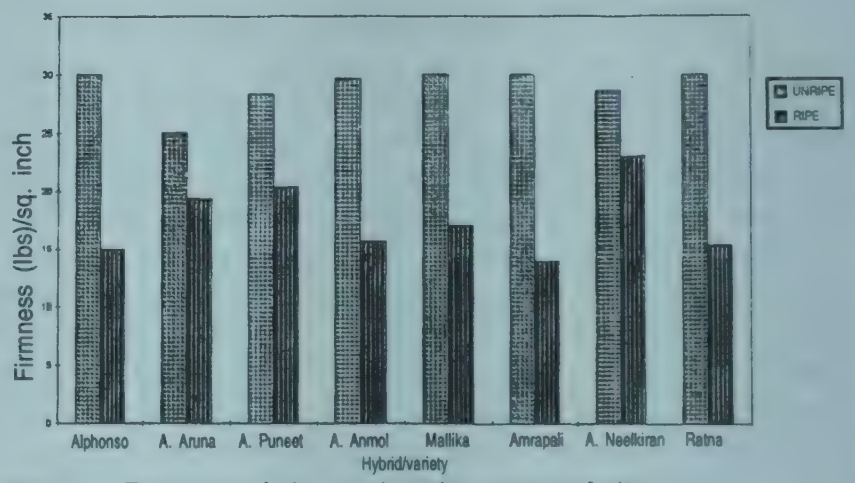

Fig. 1. Firmness of ripe and unripe mango fruits

per cent. In ripe fruits, pulp to peel ratio decreased due to decrease in pulp component with a corresponding increase in peel per cent. The peel colour of unripe mango fruit, which was light green or green or dark green turned to light yellow to orange yellow due to the breakdown of chlorophyll, leading to disappearance of green colour. The edible part of the fruit, which was white to pale yellow turned to yellow or deep yellow or orange yellow due to the development of carotenoids. Gradual increase in carotenoids and the change of pulp colour into deep yellow during ripening has also been reported by Kausik and Ranjit Kumar (1992).

Chemical composition of fruits : Significant differences were observed in the chemical constituents of mango fruits due to ripening (Table 3). The TSS of pulp was increased from 8.55 to 19.01 °Brix). This could be attributed mainly to the breakdown of starch into water soluble sugars. Krishnamurthy et al (1960) reported complete hydrolysis of starch into sucrose and glucose during ripening along with a proportional increase

TABLE 3. CHANGES IN CHEMICAL COMPOSITION OF MANGO FRUITS DUE TO RIPENING

Condition of fruit	T.S.S., °Brix	pH	Acidity, %	Reducing, sugars, %	Non-reducing sugars, %	Total sugars, %	Sugar:Acid ratio	Starch, %	Vitamin C, mg/100 g	Carotenoids, µg/100g
Unripe	8.55	2.85	2.71	1.75	0.94	2.69	0.99	10.70	40.83	498
Ripe	19.01	4.38	0.41	4.34	6.73	11.16	27.22	0.43	11.08	8071
Change	+ 10.46	+ 1.53	- 2.30	+ 2.59	+ 5.79	+ 8.47	+ 26.23	- 10.27	- 29.75	+ 7573
SEm ±	0.08	0.01	0.01	0.03	0.06	0.04	0.18	0.03	0.24	214
CD at 5%	0.23	0.04	0.04	0.08	0.17	0.13	0.51	0.08	0.69	614

in TSS. The pH of mango fruit increased considerably during ripening which could be attributed to the decline of acidity. Baqui et al (1977) noticed increased activity of citric acid glyoxylate during ripening, whereas Sathyan and Patwardhan (1983) attributed reduction in acid content to their conversion into sugars and their further utilisation in metabolic process. There were considerable increases in the reducing, non-reducing and total sugars which might be due to the breakdown of starch into sucrose, glucose and fructose (Selvaraj et al. 1989). The sugar acid ratio was 0.99 in unripe fruits and increased to 27.22, resulting in sweetness of ripe fruits. Increase in sugar: acid ratio was attributed to Increase of total sugars coupled with a steep decline of acid content. Ripening resulted in decline of starch content from 10.70%-0.43% which was attributed to the increased activity of amylase and other enzymes resulting in gluconeogenesis (Fuchs et al. 1980; Sathyan and Patwardan 1984; Selvaraj et al, 1989). The vitamin C content of unripe mango fruits was higher than the ripe fruits and the reduction due to ripening was from 40.83 to 11.08 mg/100g. Similar reduction in vitamin C content has been reported by Sahni and Khurdiya (1989) and Patil (1990) in mango varieties 'Dashehari', 'Chausa', 'Neelum' and 'Amrapali'. The one more major change that was observed in the chemical constituents during ripening was increase of carotenoids from 498 to 8071 µg/100g. Sahni and Khurdiya (1989) and Patil (1990) have also reported drastic increase in carotenoids due to ripening in mango fruits.

From the above results, it may be concluded that the major changes in mango fruits due to ripening are reduction in fruit weight, volume, length, thickness and firmness coupled with increases in TSS, pH, sugar:acid ratio and carotenoids. Reduction in acid, starch, vitamin C and pulp contents were the other changes.

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Survivability of Pathogenic *Listeria monocytogenes* Against Nisin and Its Combination with Sodium Chloride in Raw Buffalo Meat Mince

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A study was conducted to examine the anti-*Listeria monocytogenes* activity of nisin (Ambicin N) alone at concentrations of 400, 800 and 1200 IU/g and in combination with 2 % sodium chloride incorporated in raw buffalo meat mince stored at 4°C for 16 days and 37°C for 36 h. Initial microbial analysis of meat mince revealed pH, extract release volume, mesophiles and psychrophiles count as 5.76, 49 ml, 33×10^4 and 15×10^4 cfu/g of meat, respectively. All the combinations of preservatives inhibited the count of *L. monocytogenes* significantly ($P<0.05$) at both the storage temperatures when compared to the control. Addition of 2 % sodium chloride increased the efficacy of nisin against *L. monocytogenes* in all the three combinations. The degree of inhibition was more at higher concentration of nisin and lower temperature. pH in treatment groups remained significantly lower than the control groups at 4°C ($P<0.01$) and 37°C ($P<0.05$). Results indicated that the combination of food grade preservatives could be an effective listeriostatic in minced meat.

Keywords : Nisin, Sodium chloride, Growth inhibition, *Listeria monocytogenes,* Buffalo meat mince.

Listeria monocytogenes is an emerging foodborne pathogen. The association of the pathogen with meat and abattoir environments is well established (Grau and Vanderlinde 1992). In view of high prevalence of *L. monocytogenes* in raw and processed meats coupled with its capability to survive under unfavourable conditions, application of the modified and/or novel approaches for the control of this organism in the food chain has become madatory. Various approaches used are the addition of organic acids (Surve et al. 1991), bacteriocins, like nisin (Benkerroum and Sandine 1988), pediocin (Motlagh et al. 1992), and lactates (Weaver and Shelaf 1993) for retarding the growth of spoilage as well as pathogenic organisms in foods. The use of bacteriocin produced by lactic acid bacteria may provide a means to control *L. monocytogenes* in foods. Nisin, produced by some strains of *Lactococcus lactis* has been described as the best characterized bacteriocin (Donkersloot and Thompson 1990). It is capable of exerting inhibitory effect only on most of the gram-positive bacteria including *L. monocytogenes* but not on the gram negative bacteria (Chung et al. 1989). However, sensitivity of injured gram-negative bacteria to nisin has been reported (Kalchayanadan et al. 1992). It has been opined that nisin alone might not be sufficient to prevent meat spoilage because of the presence of gram-negative and other gram-positive nisin-resistant bacteria in meats (Chung et al. 1989). Addition of sodium chloride to growth medium containing nisin has been reported to probably antagonize the sporicidal action of nisin by interfering with nisin adsorption on the spores of *Bacillus licheniformis* (Bell and Delacy 1985). On the contrary, addition of 2 % sodium chloride in the medium has been found to increase the efficacy of nisin (Harris et al. 1991). The present study was envisaged to study the efficacy of three different concentrations of nisin alone and in combination with 2% sodium chloride on the survivability of pathogenic *L. monocytogenes* in raw buffalo meat mince stored at 4°C and 37°C.

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Pathogenic strain of *L. monocytogenes* MTCC 1143 (NCTC 11994) was procured from Institute of Microbial Technology, Chandigarh, India. The growth approximation of standard strain in bacterial suspension was done by McFerland Nephelometric technique (Paik and Suggs 1974) and the bacterial growth was quantified (ICMSF 1978) and adjusted to give counts of approximately 10^3 cells per 0.1 ml of the bacterial suspension.

The fresh raw buffalo meat was purchased from local retail outlets at Bareilly city, India. It was finely minced in sanitized meat mincers (Electrolux, Model 320064, Stockholm, Sweden) using 8 and 6 mm sieves. The samples each weighing 150 g were drawn from the minced meat in sterilized polythene sachets. The samples of minced meat were analyzed for extract release volume (ERV) (Shelef 1974), pH (AOAC 1975) and for total viable counts (TVC) for mesophiles and psychrophiles (ICMSF 1978). Presumptive *L. monocytogenes* counts was also carried out. For ERV, 15 g of minced meat was blended with 60 ml of distilled water in a homogeniser (Ultra-Turrax, Type TP 18/10, Janke and Kunkel, Staufen, Germany) and homogenate was immediately filtered through Whatman filter paper No. 1 of 16 cm^2 size. The volume of filtrate collected in first 15 min was recorded as ERV of meat. For estimating mesophiles, psychrophiles and presumptive *L. monocytogenes* counts, 25 g meat mince was homogenized with 225 ml normal saline solution (NSS). For mesophiles and psychrophiles counts, the homogenate was diluted serially in 10-fold dilutions in NSS and inoculated onto plate count agar and incubated at 37°C for 24-48 h and at 7°C for 10-14 days, respectively. For presumptive *L. monocytogenes* count, it was first inoculated in University of Vermont broths I and II for enrichment and then streaked onto Dominguez-Rodriguez isolation agar (DRIA) plates (Dominguez-Rodriguez et al.1984). The inoculated plates were incubated at 37°C for 48 h.

Nisin under the trade mark of Ambicin N (Lot code ZCE) was provided *gratis* by Aplin and Barret Limited (Trobridge, Wittshire, England). The activity was indicated as 37×10^6 IU per g.

The working solution was prepared as per the method described by Benkerroum and Sandine (1988). The Ambicin N was solubilised in distilled water acidified to pH 2.0 with hydrochloric acid so as to give 10000 IU per ml of solution. The solution was filter-sterilized using 0.22 μ filter and stored at -20°C.

Each of the meat sample contained in polythene sachets was treated separately with the three different combinations of nisin and sodium chloride:

Group A : 400 IU nisin per g meat; Group B: 800 IU nisin per g meat; Group C: 1200 IU nisin per g meat; Group D: 400 IU nisin per g meat + 2 % sodium chloride; Group E: 800 IU nisin per g meat + 2% sodium chloride; Group F: 1200 IU nisin per g meat + 2% sodium chloride; Control, no treatment with preservatives.

The minced meat samples in polythene sachets were inoculated with the quantified and calibrated bacterial suspension at the rate of 10^3 bacterial cells per g meat. Sachets were sealed and contents were mixed in the Stomacher (Model BA 6021, Seward Laboratory, London) for 3 min and then incubated at 4°C and 37°C for 16 days and 36 h, respectively, for each group under study.

For each group, 10 g aliquots were drawn from control and treatment groups on 1, 4, 7, 10, 13 and 16 days from inoculated samples stored at 4°C and 12, 24 and 36 h from those incubated at 37°C. Each aliquot was then processed for estimation of *L. monocytogenes* count using DRIA and determination of pH.

The data obtained were statistically analyzed using randomized block design as described by Snedecor and Cochron (1968).

The initial total viable counts (TVC) of mesophiles and psychrophiles were 33 × 10^4 and 15 × 10^4 cfu/g of meat, respectively, with no detection of *Listeria* sp. The initial pH and ERV were 5.76 and 49 ml, respectively. These parameters were within the acceptable limits, as the normal values have been reported to be 5.8 for pH and 48-53 ml for ERV (Jay 1986) less than 10 × 10^6 organisms per g for TVC of psychrophiles (Nickerson and Sinskey 1972) and 5 to 15 × 10^6 per g TVC of mesophiles (Jay 1986).

In the control group, the count of *L. monocytogenes* increased from initial 3 $\log_{10}$ cfu/g to 6.39 $\log_{10}$ cfu/g by the end of 16 days' storage at 4°C (Fig. 1). The treatment of meat with nisin alone and in combination with sodium chloride resulted in significant reduction ($P<0.05$) in the count of *L. monocy-togenes* in all the treated groups. The reduction was more pronounced in group C than groups A and B, when only nisin was added. The count of the pathogen was reduced by 0.32 $\log_{10}$ cfu/g on day 7 to 2.24 $\log_{10}$ on day 4 to more than 4.39 $\log_{10}$ on day 16 in group B, by 1.51 $\log_{10}$ on day 1 to more than 4.39 $\log_{10}$ on day 16 in group C in comparison to the control. The count of pathogen reduced below detection limits (<100 cfu/g) in group C on day 4 onwards of storage and in group B on day 13 onwards. The count of the pathogen was reduced by 0.36 $\log_{10}$ on day 16 in group D, by 1.14 $\log_{10}$ on day 1 to more than 4.39 $\log_{10}$ on day 16 in group E and

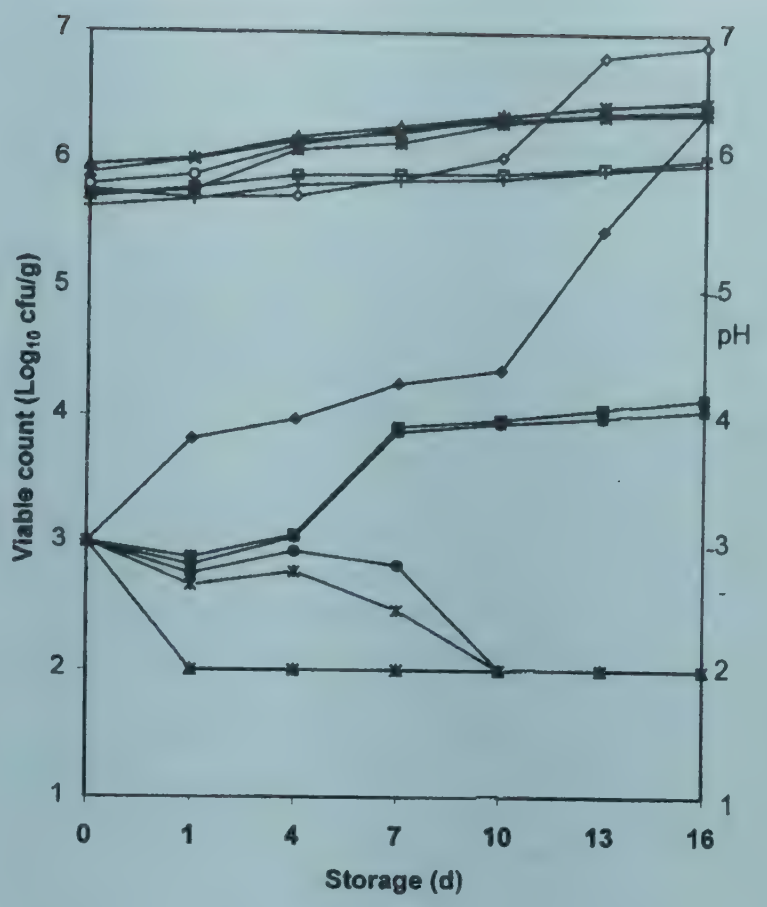

Fig. 1. Changes in the counts of *Listeria monocytogenes* and pH during storage of control and treated raw buffalo meat mince at 4°C.
-◆-, Viable count in control; -◇-, pH changes in control: -■-, Viable count in treated sample with 400 IU nisin/g; -□-, pH changes in treated sample with 400 IU nisin/g -✱-, Viable count in treated sample with 400 IU nisin/g + 2% sodium chloride; -+-, pH changes in treated sample with 400 IU nisin/g + 2% sodium chloride; -●-, Viable count in treated sample with 800 IU nisin/g; -o-, pH changes in treated sample with 800 IU nisin/g; -✱-,Viable count in treated sample with 800 IU nisin/g + 2 % sodium chloride, -✱-, pH changes in treated sample with 800 IU nisin/g + 2% sodium chloride;-▲-,Viable count in treated sample with 1200 IU nisin/g; -△-, pH changes in treated sample with 1200 IU nisin/g;-X-, Viable count in treated sample with 1200 IU nisin/g+ 2% sodium chloride; -X-, pH changes in treated sample with 1200 IU nisin/g + 2% sodium chloride

more than 1.81 $\log_{10}$ on day, to more than 4.39 $\log_{10}$ on day 16, as compared to the control. The patterns of growth inhibition of *L. monocytogenes* observed in groups added nisin alone and in combination with sodium chloride were similar. However, degree of inhibition was more pronounced in combined treatment. The observed inhibition of the pathogen by nisin is in accordance with the observations that nisin reduces the count of pathogen on surface of meat dipped in a solution containing 10^4 IU/ml (Chung et al. 1989), in broth containing 10 μg/ml (Harris et al. 1991) and in non-sterilized cottage cheese containing 2.55 × 10^3 IU/ml (Benkerroum and Sandine 1988). The increase in degree of inhibition by nisin in combination with sodium chloride is in accordance with that of Harris et al (1991), who found that addition of sodium chloride to the medium containing nisin increased the listericidal effect of nisin, particularly at nisin concentrations of less than 10 μg/ml. Although sensitivity of nisin was enhanced by addition of 2% sodium chloride, a concentration of 2.5%

in the medium was necessary to observe a significant inhibition (Harris et al. 1991). It was opined that common salt might have made *L. monocytogenes* populations more susceptible to nisin by placing additional environmental stresses on the organism. However, more direct effects were also possible.

In the present study, the treatment of meat with nisin alone and in combination with 2 % sodium chloride brought a noticeable reduction in pH of meat, which remained significantly ($P<0.01$) lower than the control group (Fig.1) throughout the 16 days storage period at 4°C. Vulneribility of *L. monocy-togenes* to the action of nisin at low pH has been reported (Benkerroum and Sandine 1988). Mohamed et al (1984) observed an enhanced effect of nisin at lower pH and attributed the effect to increased stability of the nisin molecule at the lower pH. Also, the probability of survival of *L. monocytogenes* has been reported to reduce significantly at lower pH (Parente et al. 1998).

At 37°C, the *L. monocytogenes* count increased rapidly from an initial 3 $\log_{10}$ cfu/g to 6.93 $\log_{10}$ cfu/g of meat at the end of storage in the control group (Fig. 2). Treatment of meat with nisin alone and in combination with sodium chloride showed significant ($P<0.05$) listeriostatic effect. The inhibitory effect was more pronounced in group C, where counts of the organism were reduced by 3.02 logs at 12 h to 4.27 logs at 36 h. The growth of the pathogen in groups A and B showed a marked initial reduction by 1.35 and 1.69 logs at 12 h, respectively, as compared to the control. The reduction was slightly increased in these groups throughout the storage and turned out to be more than 2.2 logs at the end of storage period as compared to the control. However, in group C, a greater initial reduction by more than 3 logs cfu/g was noticed at 12 h, which remained almost static up to 24 h and then increased further to a maximum of 4.26 logs at the end of storage period as compared to the control. The patterns of the growth inhibition of *L. monocytogenes* were similar to that observed with respective concentrations of nisin alone after treatment of meat with nisin in combination with sodium chloride. However, the growth inhibition was increased from 0.02 to 0.11 log in group D, 0.11 to 0.27 log in group E and 0.05 to 037 log in group F when compared to groups A, B and C, respectively.

pH of the control sample showed a consistent increase from an initial value of 5.76 to 7.75 at the end of storage at 37°C (Fig. 2). On the contrary, pH values of all the treated groups were significantly ($P<0.05$) low and showed a decrease of more than 1.94 units at the end of the storage period in comparison to the control. While comparing of pHs within the groups, a rise of 0.47 units in group A and about 1.0 unit in rest of two groups was noticed during the storage period, whereas, rise in the pH of control was more than 2.0 units during the period. However, comparing of pH among the three groups treated with nisin alone, it was found to be maximum in group C with an increase of 0.16 to 0.19 unit over group B. The data indicated that pH of *L. monocytogenes*-inoculated meat increased with the increase in concentration of pure nisin added to it. Addition of 2% sodium chloride in combination with nisin reduced the pH of meat slightly as compared to the addition of nisin alone.

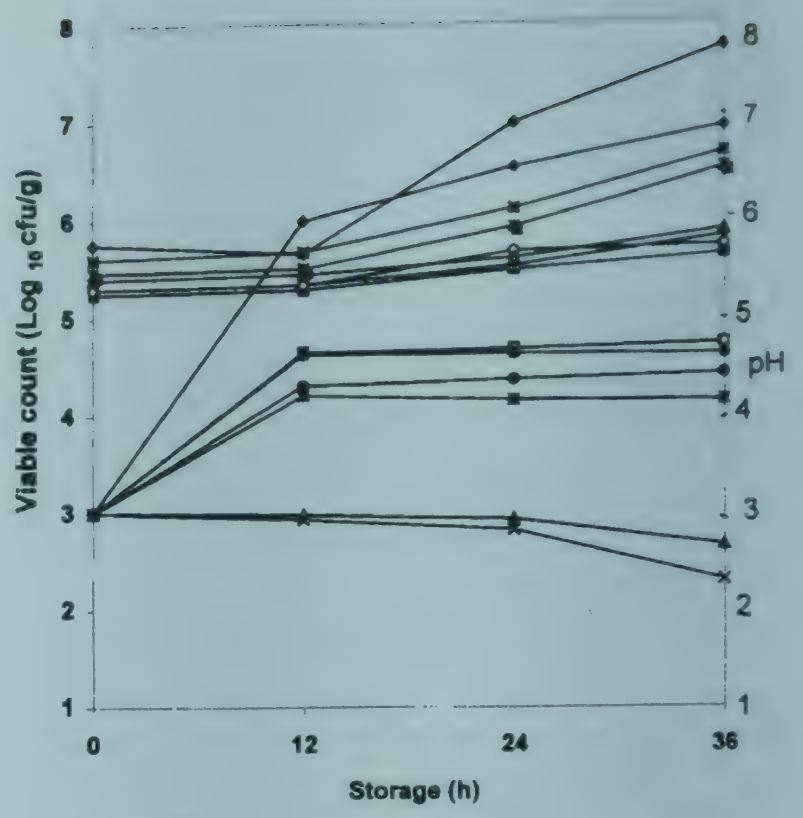

Fig. 2. Changes in the counts of *Listeria monocytogenes* and pH during storage of control and treated raw buffalo meat mince at 37°C.
-◆-, Viable count in control; -◇-, pH changes in control: -■-, Viable count in treated sample with 400 IU nisin/g; -□-, pH changes in treated sample with 400 IU nisin/g -+-, Viable count in treated sample with 400 IU nisin/g + 2% sodium chloride; -+-, pH changes in treated sample with 400 IU nisin/g + 2% sodium chloride; -●-, Viable count in treated sample with 800 IU nisin/g; -o-, pH changes in treated sample with 800 IU nisin/g; -X-,Viable count in treated sample with 800 IU nisin/g + 2 % sodium chloride, -X-, pH changes in treated sample with 800 IU nisin/g + 2% Sodium chloride;-▲-,Viable count in treated sample with 1200 IU nisin/g; -△-, pH changes in treated sample with 1200 IU nisin/g;-X-, Viable count in treated sample with 1200 IU nisin/g+ 2% Sodium chloride; -X-, pH changes in treated sample with 1200 IU nisin/g + 2% sodium chloride

The inhibitory effect of nisin on pathogen observed in all the three groups at 37°C was less pronounced than that observed in the respective groups at 4°C. At low temperature, conservation of effectiveness of nisin for a longer time (Gibbs and Hurst 1964) and decrease in nisin activity at ambient temperature (Chung et al. 1989) has been reported.

In the present study, the initial microbial count of the meat mince was within the acceptable limits and the addition of 400, 800, or 1200 IU/ml of nisin per g of meat alone or in combination with 2% sodium chloride was found to exert a significant inhibitory effect on pathogenic *L. monocytogenes* at both the storage temperatures. The inhibition of pathogen increased with the concentration of nisin and decreased with the increase in storage temperature. It may be concluded that nisin in combination with 2% sodium chloride can be used as an effective listeriostatic agent in ground beef stored at refrigeration temperature.

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RESEARCH NOTE J. Food Sci. Technol., 2001, Vol. 38. No. 2, 142-144

Chemical Quality of *Paneer* Prepared from Milk Added with Urea

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Quality of *paneer* from standardized buffalo milk containing 0.05 and 0.1 % of added urea was evaluated with respect to yield, sensory characteristics, gross chemical composition and other chemical indices. About 14.4, 31.2 and 50. 4 mg urea/100g appeared in *paneer* (Control, U_1 and U_2) prepared from milk containing 0, 0.05 and 0.10% of added urea, respectively. Urea treated milk had shown lower yield of U_1 and U_2 *paneer* by 4.2 and 9.5 %, respectively as compared to control milk. The sensory properties of *paneer* had not much affected by the addition of urea to milk, but U_2 *paneer* showed hard body with dry surface. A significant ($P<0.05$) lower pH and higher titratable acidity, water-soluble N and free acidity contents were observed in both U_1 and U_2 *paneer* samples. On the other hand, urea had no marked influence on gross chemical composition except for total protein and free fatty acid contents in *paneer*. Chemical changes in *paneer* samples prepared from urea-added milk during storage was less pronounced as compared to control *paneer*. On the basis of residual urea and water soluble N contents in *paneer*, it was possible to explain for its source from milk used in its preparation.

Keywords : *Paneer*, Urea, Yield, Chemical characteristics, Storage.

Urea is universally known to be a nitrogenous fertilizer, essential for the plant growth. It is also a natural constituent of normal body fluids including blood (160-420 mg/litre), urine (5000-12000 mg/litre) and milk (100-450 mg/litre) [Miller 1971]. Histopathological study of visceral organs, stomach, intestine and adrenals of rabbits showed that when diet containing 0.5, 1.0 and 1.5 % urea was administered for 180 days, tissue lesions involving cell proliferation, degeneration and necrosis were pronounced in the liver and kidney of rabbits (Krishna et al. 1990). Addition of urea to milk is not permitted under PFA Act (1984). However, there are a few unscrupulous vendors, who deliberately add urea to milk to increase the SNF content and/or to improve the heat stability of milk. Urea is also used as one of the ingredients in the preparation of so-called "synthetic milk". Such urea-added milk is often processed for the manufacture of various indigenous dairy products. The present investigation was carried out to study the effect of addition of urea to milk on the yield and chemical quality of *paneer* prepared from such milk, since no information is available on this aspect.

Preparation of paneer : Composite raw fresh buffalo milk and fresh skim milk were collected from the cattle yard and Experimental Dairy of the Institute, respectively. The milk was standardized to fat: SNF ratio of 1:1.65 and was divided into three equal parts. Out of these, two parts were treated with solution of urea at 0.05 and 0.1 % of milk, respectively, and the remaining part was used as control (without urea addition). From each part (2 kg), the *paneer* was prepared in the laboratory under standard conditions as described by Sachdeva and Singh (1998) and stored at refrigeration temperature (10±2°C) till analysis.

Analytical procedures : Standardized buffalo milk used for *paneer* making was analyzed for moisture, fat, total proteins, lactose, ash, pH and titratable acidity as per BIS (1981), non-protein N (soluble in 12 % trichloroacetic acid) using Kjeldahl method and free fatty acids (Thomas et al. 1954). Sensory evaluation of raw *paneer* was carried out for flavour, body, texture and appearance by a panel of five judges as described by Patil and Gupta (1986). The stored *paneer* was analyzed for residual urea (Bector et al. 1998), pH (Roy 1990) and other chemical characteristics as described by Nayak and Bector (1998). The data obtained were analyzed for ANOVA (Snedecor and Cochran 1989).

Paneer *yield :* The yields of U_1 and U_2 *paneer* decreased from 22.65 to 21.7 and 20.5%, on addition of 0.05 and 0.1 % urea to milk , respectively and decreased by about 4.2 and 9.5%, respectively, as compared to control (Table 1). The decrease in yield of *paneer* prepared from milk containing 0.1% of added urea was significant ($P<0.05$). However, the decrease in yield of *paneer* prepared from milk containing 0.05 % of added urea was not significant. Further, the recovery of total solids was lower in U_1 and U_2 *paneer* samples (65.48 and 62.06 %, respectively) as compared to control *paneer* (67.04 %). The low yield of *paneer* prepared from urea-added milk might be due to the low recovery of total solids and more expulsion of whey from curd during pressing. Guinot-Thomas (1992) also reported a small decrease in the yield of Camembert cheese when milk was artificially supplemented with urea at a concentration of 1 g/litre.

Residual urea content : The natural urea content of standardized buffalo milk was 21.0 ± 3.32 mg/100 ml (Table 1). A part of this natural urea (about 15.53 % of milk urea) appeared in *paneer* (14.4 mg/100g), which increased to 20.5 and 28.8 mg/100 g on the 5 th and 10 th days of storage, respectively. The addition of 0.05 % urea to milk resulted in residual urea concentration (31.2 mg/100 g) and worked out to be a recovery of about 9.54 %, which was further increased to 50.8 and 72.0 mg/100 g *paneer* on the 5 th and 10 th days of storage, respectively. Similarly, addition of 0.1 % urea to milk increased the residual urea (50.4 mg/100 g) and gave a recovery of about 8.54 %, which on storage, increased to 71.0 and 96.0 mg/100 g of *paneer*, respectively. This increase in urea content could be due to the decomposition of milk proteins during storage into various compounds and urea may be one of them.

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TABLE 1. EFFECT OF ADDED UREA TO MILK ON THE CHEMICAL CHARACTERISTICS OF *PANEER* STORED AT 10 ± 2°C

Chemical characteristic	Stand. milk	Storage period, days: Control 0	Control 5	Control 10	U_1 0	U_1 5	U_1 10	U_2 0	U_2 5	U_2 10	CD (P<0.05)
Yield, %	-	22.65[a] ± 0.63	-	-	21.70[a] ± 0.88	-	-	20.50[b] ± 0.32	-	-	1.39
Moisture, %	84.10 ± 0.09	52.93 ± 0.61	52.74 ± 0.63	52.73 ± 0.51	52.02 ± 0.32	51.99 ± 0.33	51.36 ± 0.17	51.87 ± 0.49	51.80 ± 0.35	51.22 ± 0.28	1.38
Fat, %	6.05 ± 0.03	25.60[a] ± 0.57	25.85 ± 0.57	25.97 ± 0.52	26.32[ab] ± 0.14	26.36 ± 0.20	26.18 ± 0.20	26.65[b] ± 0.13	26.58 ± 0.19	26.77 ± 0.13	1.04
Total proteins, %	4.15 ± 0.09	17.26[a] ± 0.28	17.26 ± 0.26	17.22 ± 0.22	17.83[ab] ± 0.45	17.89 ± 0.23	17.75 ± 0.30	18.45[b] ± 0.36	18.54 ± 0.16	18.47 ± 0.25	1.09
Lactose*, %	4.90 ± 0.02	2.60 ± 0.15	2.51 ± 0.15	2.21 ± 0.19	2.61 ± 0.15	2.45 ± 0.10	2.16 ± 0.10	2.69 ± 0.03	2.54 ± 0.12	2.22 ± 0.14	0.43
Ash %	0.77 ± 0.01	1.57 ± 0.07	1.62 ± 0.08	1.87 ± 0.18	1.62 ± 0.04	1.72 ± 0.19	1.85 ± 0.14	1.71 ± 0.04	1.87 ± 0.20	1.93 ± 0.28	0.17
pH* at 20°C	6.72 ± 0.01	5.80[a] ± 0.01	5.68 ± 0.01	5.40 ± 0.015	5.62[b] ± 0.015	5.58 ± 0.01	5.40 ± 0.01	5.56[b] ± 0.01	5.42 ± 0.15	5.30 ± 0.01	0.07
Titratable acidity*,% (as lactic acid)	0.152 ± 0.004	0.550[a] ± 0.004	0.565 ± 0.016	0.990 ± 0.011	0.645[b] ± 0.011	0.745 ± 0.029	0.990 ± 0.012	0.660[b] ± 0.013	0.760 ± 0.021	1.070 ± 0.026	0.09
Water soluble N*,%	0.045♦ ± 0.002	0.070[a] ± 0.003	0.112 ± 0.002	0.145 ± 0.001	0.120[b] ± 0.002	0.140 ± 0.007	0.212 ± 0.011	0.210[c] ± 0.002	0.212 ± 0.007	0.230 ± 0.20	0.09
Free fatty acids*,% (as oleic acid)	0.034 ± 0.001	0.060 ± 0.005	0.068 ± 0.002	0.130 ± 0.001	0.070 ± 0.002	0.090 ± 0.006	0.141 ± 0.011	0.068 ± 0.002	0.096 ± 0.007	0.141 ± 0.020	
Free acidity*,% (as oleic acid)	-	0.20[a] ± 0.01	0.26 ± 0.05	0.35 ± 0.02	0.32[b] ± 0.02	0.32 ± 0.05	0.42 ± 0.01	0.32[b] ± 0.02	0.32 ± 0.02	0.44 ± 0.01	0.03
Residual urea (mg/100 g)	21.00 ± 3.32	14.40 ± 1.39	20.50 ± 0.55	28.80 ± 0.34	31.20 ± 0.45	50.80 ± 0.49	72.00 ± 0.27	50.40 ± 0.35	71.00 ± 0.10	96.00 ± 0.50	-

U_1 and U_2 indicate *paneer* prepared from milk containing 0.05 and 0.1 % added urea, respectively
Data represent mean ± S.E. of three replicates
Values bearing different superscripts in each row differ significantly (P<0.05)
* Storage effect was different significantly (P<0.01)
♦ indicates non-protein N content

Sensory evaluation : The sensory scores for flavour, body and texture and appearance of test *paneer* samples were compared with those of control *paneer* (Table 2). The appearance and flavour of all *paneer* samples were similar. However, the body and texture scores were less for both U_1 and U_2 *paneer* samples than those of control *paneer* and particularly U_2 *paneer* showed hard body with dry surface. During storage, control and test *paneer* samples were found to be free from any objectionable flavour and there was no change in appearance up to 5 days. A slight sour smell with dull appearance was noticed in control and test *paneer* on the 6th day. A yellow slimy layer with distinct off-flavour was observed in all *paneer* samples on the 10 th day indicating spoilage of *paneer.* Hence, this was considered as cut-off period for analysis of samples.

Chamical characteristics : The total proteins (calculated on the basis of total N) and water-soluble N content of *paneer* progressively increased with increase in the rate of urea addition to milk (Table 1). The increase being significant over the control *paneer* and of higher magnitutde in U_2 *paneer*, could be due to the higher residual urea content in test *paneer.* The lower moisture contents in test *paneer* samples as compared to control *paneer* revealed a negative impact of urea addition to milk in higher proportion on the yield of

TABLE 2. EFFECT OF ADDED UREA TO MILK ON SENSORY CHARACTERISTICS OF FRESH *PANEER*

Attribute	Max. score	Average secured score: control	U_1	U_2
Flavour	50	47	47	47
Body and texture	35	34	32	28
Appearance	10	9	9	8
Package	5	4	4	4
Total	100	94	92	87

U_1 and U_2 indicate *paneer* prepared from milk containing 0.05 and 0.1% added urea, respectively

paneer. Such decrease in moisture content of U_2 *paneer* led to a hard body with dry surface. Urea levels had no significant effect on free fatty acids, lactose and ash contents. However, urea had significant (P<0.05) influence on pH, titratable acidity and free acidity of *paneer.* The lower and higher values of pH and titratable acidity and free acidity, respectively, in test *paneer* may be ascribed to the use of higher amount of coagulant and higher total solids of test *paneer,* because addition of urea to milk increased its heat stability (Mehanna et al. 1986). All the stored *paneer* samples had significantly lower lactose and pH as well as higher titratable acidity, water-soluble N, free fatty acids and free acidity when compared

with their respective fresh *paneer* samples. These changes may be due to proteolytic and lipolytic changes taking place in the *paneer* samples during storage (Haridas and Nayarayanan 1976). From critical observation, it was apparent that there was a slight change in chemical parameters during the first 5 days of storage, but appreci-able change was noticed in *paneer* stored for 10 days. Further, these chemical changes in both U_1 and U_2 *paneer* samples could be considered slower as compared to control *paneer.*

From the present study, it may be concluded that addition of urea to milk progressively declined the yield and sensory quality of *paneer* with increase in its concentration and also changed the chemical characteristics of *paneer.* As residual urea and water-soluble N contents of *paneer* depend on the urea content of milk, this can explain the presence/absence of extraneous urea in *paneer.*

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RESEARCH NOTE

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Influence of Lactoserum on the Corrosion of 'AISI 304' Stainless Steel

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Experimental studies on the effect of a whey solution on 'AISI 304' stainless steel (304 SS) were carried out. Conditions in cheese-manufacturing were simulated and electro–chemical measurements of corrosion, as well as variations in pH and electrolyte acidity were determined. Surface damage was characterized with scanning electron microscopy (SEM). The results obtained were used to evaluate whey exposure times versus stainless steel corrosion susceptibility and its relationship with contamination of the medium.

Keywords : Characterization, Degradation, Lactoserum, Whey.

Corrosion is becoming important because of the extensive use of expensive metal equipment in food industry, which has to be replaced or repaired due to impairment caused by the operation environment [IDF 1987; Zumelzu and Cabezas 1995 a).

In the dairy industry, material corrosion can be the source of chemical contamination of milk and its derivatives in the form of iron and copper ions. Also, corroded metal surfaces make the cleaning and sanitizing processes much harder, contributing in this way to the microbiological-induced corrosion (MIC) (Zumelzu and Silva 1996; Zumelzu and Cabezas 1996).

Whey is a subject under permanent research, being the major by-product of the conversion of milk into cheese, casein or casein derivatives. Lactose is one of the main solid components, which is a carbonhydrate, characterized by its low edulcorant power and relatively low solubility.

Because of its characteristcs and processing variables (Dupeyrat and Labbe 1987; Mathews 1984), whey affects the surface of stainless steels, inducing microcorrosion and deterioration of finishing quality, due to the high lactoserum's biochemical demand of O_2, causing contamination in the containers.

Hence, cheese whey is defined as "the fluid resulting from the coagulation or acidification of milk, after the segregation of casein and the major part of fat". It is mainly composed of water, lactose, proteins, minerals and lactic acid, which under certain processing conditions can favour localized corrosion on 'AISI 304' stainless steels (Zumelzu 1989; Daufin and Labbe 1987).

Stainless steels are of common use in the manufacturing of food industry equipment, because of their high resistance to chemical reactions and high temperature exposure.

The present work was undertaken to study the performance of 'AISI 304' stainless steel in a whey solution, partially simulating the manufacturing process of cheese concerning the metal surface interface with the electrolyte.

The working hypothesis was : "Routine pH and lactic acid tests are insufficient to characterize the degradation processes of the AISI 304 stainless steel-lactoserum system in time." The other hypothesis was: "Combination of the former trials with current density measurements at zero polarization and observations of the solid's surface by scanning electron microscopy allow to adequately characterize the evolution of the degradation processes in time."

The purpose of this study was to evaluate the characteristics and magnitude of the deterioration of steel during 72 h of contact with lactoserum, since this deterioration has consequences on the efficiency of the cleaning and maintenance processes of devices built with this type of steel, normally used in the milk industry, in adequate sanitizing conditions.

Corrosion resistance of steel to whey was determined by means of experimental assays and characterized through scanning electron microscopy for the surface damage undergone.

Influence of lactoserum on stainless steel corrosion : The testing material employed during this trial was 'AISI 304' (304 SS) stainless steel (Fe, 0.08 C, 0.5 Si, 1.3 Mn, 17.8 Cr, 8.2 Ni) and the electrolyte used was based on whey, whose characteristics are indicated in Table 1.

The electrolyte was prepared with 25 g of powder lactoserum manufactured by the Centro Technológico de la Leche UACH (Groseclose 1984), dissolved in 2500 ml of pasteurized water (80°C).

The steel samples used were cut according to the dimensions and shape of the teflon sample holder, leaving an exposed area of 2 cm^2. Then, the plastic protection layer was removed and the samples were washed in acetone and distilled water and placed on the sample holders. The following step was applied to make measurements every 12 h, during a 72 h test period; the test was performed on an open circuit with no agitation, dipping the samples into a bath containing lactoserum at 25°C.

The electro-chemical arrangement included a saturated colomel electrode as a control parameter, whose ionic terminal

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TABLE 1. COMPONENTS AND MAIN COMPOSITION OF TESTED WHEY

Components	Percent	Percent of dry matter
Water	94.25	-
Proteins	0.80	13
Lactose	4.30	76
Ash	0.55	10
Fat	0.10	1

TABLE 2. STABILIZATION POTENTIAL, CORROSION DENSITY, pH, ACIDITY AND LACTIC ACID OF TESTED WHEY

Time, h	Stabilization potential, mV(SCE)	Corrosion density, mA/Cm²	pH	Acidity °A,	Lactic acid, %
00	-111	-0.040	5.88	21	0.189
12	-105	0.016	5.96	22	0.180
24	-121	0.048	5.90	19	0.171
36	-113	0.027	5.88	19	0.171
48	-153	0.027	5.96	21	0.189
60	-129	0.018	5.95	23	0.208
72	-102	0.055	5.96	23	0.208

was connected to a Luggin capillary and a helicoidal platinum counterelectrode, both located in chambers separated by a porous fritting (Zumelzu and Cabezas 1995 b). To the cell with a capacity of 250 ml was added 50 ml of lactoserum and the standard calomel electrode and counterelectrode were placed at a distance of 2 mm and 3.5 mm from the work electrode, respectively. After reaching the stabilization potential, by a PINE RDE4 potentiostat, linear scannings of the potential were performed up to a maximum of + 300 mV, at a speed of 0.2 mV/sec, following the same procedure of earlier studies (Zumelzu and Cabezas 1995 b Zumelzu 1989).

The pH of this solution was measured in a Hanna HI 8414 microprocessor and the acidity was determined by titration of 10 ml with a solution of NaOH, according to the NcH 1738 Chilean standard. The selection of pH as a parameter was made in terms of its practical use as a measurement tool and the study of its potential as a predictor.

Microstructural observations of samples were made with a scanning electron microscopy (Hitachi SEM) for each testing time in the whey solution electrolyte.

Corrosion processes and damage morphology in stainless steel : Table 2 shows the data on 12 h measurement intervals concerning the stabilization potential for the saturated calomel electrode, corrosion density obtained by analytical treatment of polarization curves in terms of Tafel lines, pH and lactic acid percentage present in whey.

As shown in Table 2, both the stabilization potential and pH are time-independent in their behaviour. The average value for the stabilization potential was of -119.14 mV (SCE) (standard deviation 15%) and the whey solution's pH showed a value of 5.93 (standard deviation 0.69%). It is difficult to imagine, as these results suggest, that both the solid-liquid interface and the composition of whey will retain their characteristics as steel-whey contact time elapses. Most probably, they will not allow to observe the complex corrosion processes at the interface because of their fundamentally macroscopic nature. The first is related to the electric structure of the interface after approximately 3 h of stabilization and the second is related to the presence of hydrogen ions in the medium, not necessarily at the interface, where the charge transference processes of corrosion effectively take place.

The analysis of the lactic acid percentage and corrosion current density showed different results. The acidity remained unchanged during the first 48h, with an average value of 0.180 (standard deviation 0.008%, falling into an acceptable range for the allowed standard (0.144-0.189). However, it increased significantly up to 0.208 after 60 h. As stated in the literature (Zumelzu 1989; Daufin and Labbe 1987), the presence of lactic acid from almost the beginning of the trial could be related to localized corrosion developing into another attack mode, which became macroscopically evident only after 60 h. The current density values observed during this trial suggested a more complex process. Three peak values, with a significant decline after a given period of time, were detected. At time 0, the first maximum value was recorded, corresponding to the initial isolated, pitting with premature damage of the passivation layer of steel. A decrease after the first 12 h was noticed, possibly due to the inhibiting effect of corrosion products, which would gradually restrain the primary in-depth growth of pitting observed during the first stage. At 24 h of the trial period, a second peak was recorded, with lower values at the time corresponding to 36, 48 and 60 h. This behaviour could be related to a second corrosion mode consisting of the coalescence of inital pitting, determining the presence of non-lacalized pitting corrosion on the passivated metal surface, leading to an increase in the internsity of the attack and giving rise to surface metal dissolution. Finally, the third peak value could be interpreted in terms of a different corrosion mode, which remained undercovered by the localized attack and its later spreading.

Fig. 1 shows the behaviour of lactic acid and corrosion current density versus time, bearing in mind that only they can validly explain the corrosion process of steel under the action of whey.

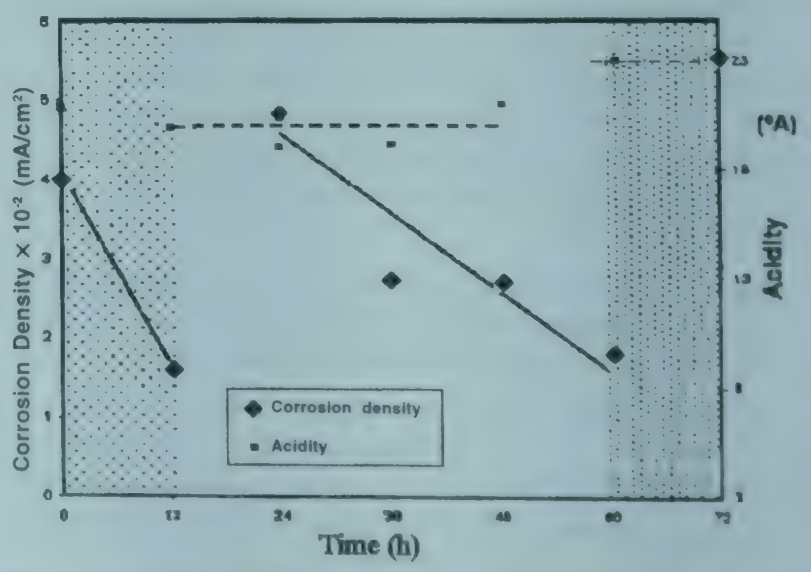

Fig. 1. Corrosion density variation (i) and acidity (°A) versus time, at a stabilization potential of-119.14 mV (SCE) ± 15%

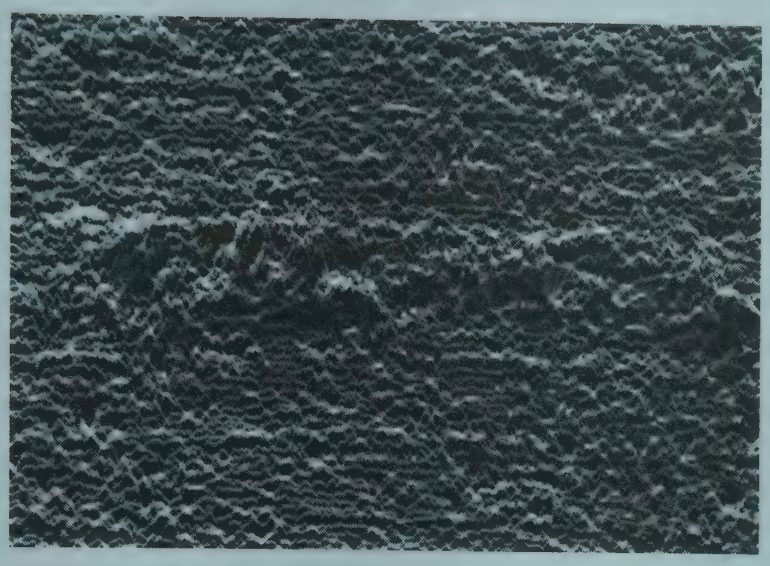

Plate 1. Sample 24 h. Modulation in "Y" with surface topography, pit corrosion with a verage diameter of 18 micra, X 800 SEM

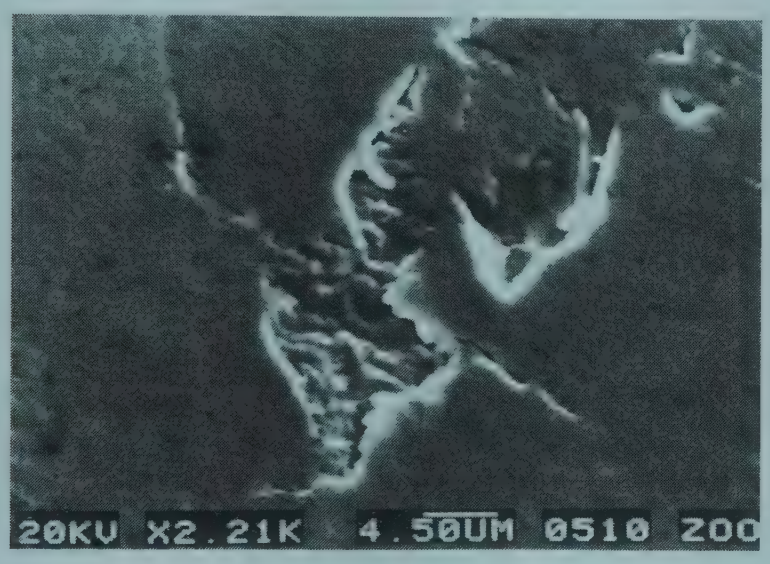

Plate 2. Sample 36 h. Depassivation and pitting corrosion on surface (dark zone), X 2.210 SEM

Scanning electron microscopy observations allowed the examination of surface deterioration in samples, making evident the presence of localized pitting corrosion at given points of the passivated meatal surface (Plate 1), when applying secondary electrons on the sample and after Y-axis modulation to show the change in morphology caused by the pit. Localized micro-corrosion increased with time after the coalescence of pits, leading to a greater extent of damaged surface and expressed as an eroded and wrinkled surface (Palte 2). This pitting micro-corrosion turned out to be severe at 60 h, with more intense surface damage caused by the rupture of the natural passivation layer of stainless steel (Cr 203), making it more susceptible to electrolyte attack (Plate 3). Plate 4 shows evidence of another current corrosion mechanism detected at 72 h in the form of localized intergranular attack moving onto the grain boundaries (dark zone) and leaving grains isolated on the surface (light zone).

From the above results, it may be concluded that the electrochemical methods and SEM observations carried out, experimentally independent, are in coincidence with their description of the degradation processes of the surface of stainless steel by the action of lactoserum, specifically the lactic acid present in the medium.

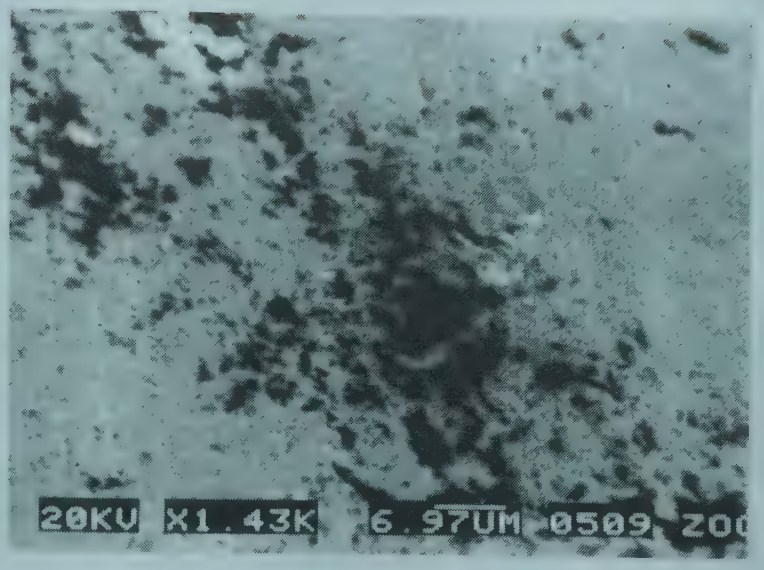

Plate 3. Sample 60 h. Severe pits corrosion, damage morphology caused by the electrolyte, X 1430 SEM

Plate 4. Sample 72 h. surface intergranular corrosion on SS304SEM

Further, the corrosion process of low-carbon AISI 304 stainless steel induced by lactoserum, developed during the 72 h study, includes a mechanism of initial localized pitting, which becomes more intense and widespread with time, finishing in an intergranular attack of the metal surface. The result of this attack is expressed as an eroded and wrinkled metal surface, significantly changing the original characteristics of the surface as to the mirror-polish finishing, necessary to sustain the basic industrial sanitizing conditions. Even though the magnitude of the solid's dissolution is not catastrophic, it is significant from the point of view of the final morphology observed on the surface of the solid, susceptible of retaining fat, which is hard to remove, thus making the cleaning process more difficult. On the basis of the coincidential description of the corroding action of lactoserum on AISI stainless steel, it can be stated that the combination of the three methods (i.e., acidity measurement techniques or percentage of lactic acid present, corrosion current density assessment and SEM observations) employed during this study can be employed to describe and evaluate these phenomena and therefore, tenable to be used in the dairy industry.

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Evaluation of Performance of Shea Fat as a Shortening in Breadmaking

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The effect of crude shea fat as a shortening in breadmaking was investigated. Margarine was used as a control fat. The straight dough method of breadmaking was used. Proofing of dough was carried out for a maximum height of 13 cm. The loaf weight (448-450 g), volume (856-860 ml) and specific volume (1.91 ml/g) were similar for breads with control fat and shea fat, respectively. Sensory evaluation of breads indicated that both samples of bread had uniform fine crumb grain, soft texture and typical flavour and taste. There was no significant difference ($p>0.05$) in terms of overall acceptability between the two sets of bread. The results suggested that crude shea fat could be used as a shortening in breadmaking.

Keywords : Shea fat, Shortening. Breadmaking, Specific volume, Sensory evaluation.

Bread is one of the convenient breakfast foods, consumed worldwide. The main ingredients for making bread are wheat flour, yeast, sugar, salt, fat and water. The fat is also called shortening, which is marketed under different trade names such as holsum, breaden, etc,. Addition of optimum amount of fat improves the loaf volume and crust and crumb characteristics of bread. However, excessive use of fat produces a close "chessy" crumb, which is soft and greasy to touch (Chung et al, 1982).

Shea fat is "butter" obtained from the kernel of *Butyrospermum paradoxum* tree, which grows throughout Savanna area of West Africa. In countries such as Ghana Togo, Benin, Mali, Burkina Faso, Cote d'voire and Nigeria, shea fat is not only used widely as a cooking medium but also has uses in cosmetic and pharmaceutical industries. In some parts of Nigeria, shea fat is also used in the treatment of minor bone dislocation and cough (Badifu 1989). Its by-products viz., the pulp and the shell make water-proof materials used on the walls of huts, farmers spread them as fertilizers or burn them as fuel. Shea fat can also be used as a cocoa butter substitute for coating in chocolates and in the cosmetic industry. The fruit is a berry, which is eaten and the kernel is covered with a hard brownish shell. Fat from the kernel is obtained by solvent extraction (Kar and Mital 1981). The fat is also obtained by traditional processing method. This involves warming up the kernel by the application of mild heat, followed by pounding in a mortar with pestle to a coarse brown paste. The paste is further ground with a large stone to a greasy mass. The fat is expelled by boiling the greasy mass with water. The fat, which floats on the water surface is skimmed off for use.

The costs of conventional shortening such as holsum, margarine are high for an average baker, whose bakeries are situated in rural and urban poor areas of Nigeria. Consequently, there is a need to search for a complementary or alternative source, which may be relatively cheap with a simple technology of production in rural settings. The chemical and physical properties of shea fat have been reported (Mital and Dove 1971; Badifu 1989; Badifu and Abah 1998). In spite of the potentials of shea fruit, not much attention has been paid to the establishment of plantation of the tree in Nigeria and parts of other countries, where the trees are growing wild in the forests. This could be due to dearth of information in the literature on its food potentials. The purpose of this investigation was to determine the effect of crude shea fat used as shortening in breadmaking on the qualities of the bread and its acceptability.

Baker's grade wheat flour, margarine (Blue band from Lever Brothers plc, Nigeria), yeast (Baker's brand), salt and sugar were purchased from a local market in Makurdi township of Benue State, Nigeria. The shea fat was supplied, on request, by a traditional processor in Makurdi.

Laboratory process of breadmaking : The straight dough method of breadmaking was used. Two batches of bread were made, one batch contained crude shea fat, which served as shortening and the other batch had margarine as shortening. The batch with margarine served as a control. The proportions of the ingredients were 210 g flour, 5.6 g yeast (2.7%), 3.5 g salt (1.7%) 5 g fat (2.4%), 15 g sugar (7.1%) and 126 ml water (60%). Yeast and sugar were put in 60 ml warm water 37-40°C) and allowed to stand for 15 min. The ingredients were added in a dough mixer and mixed to form dough. The dough was put into a baking pan greased with shea fat or margarine accordingly and covered with greased bread wrapper. This was allowed to ferment at 35 ± 1°C and 75% relative humidity for 45 min. This was further allowed to proof at 36-42°C and 80% relative humidity for 25 min. The proofed dough in pan was charged into a free-air oven at 240±5°C for 35 min to produce bread.

Proximate composition : Protein (N × 6.25), fat, fibre, moisture and ash contents of the flour and bread were determined according to AOAC (1984) methods. The carbohydrate was calculated by difference.

The fatty acids of the flour and shea fat were converted to their methyl esters with sodium methoxide according to the procedure of Glass and Troolin (1965). The fatty acid methyl esters were determined using a GLC under the operating conditions previously described by Badifu (1989). Sugars were

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extracted from the flour as described by Figueroa and Khan (1991). The flour (50 mg) was added to 1 ml of 0.05 M acetic acid, vortexed for 10 min and then centrifuged at 12,000 × **g** for 10 min. The supernatant was decanted and an aliquot (50 ml) was diluted with 950 ml of distilled water and used for the determination of sugars. Sugar content was estimated by the phenol-sulphuric acid method of Dubois et al (1956), in which i ml of sugar extract was treated with 1 ml of 5% solution of phenol and 5 ml of concentrated H_2SO_4. After 20 min, the absorbance was read at 490 nm. The amount of sugar in the flour was estimated from a standard curve prepared with D-glucose (Sigma). Fat absorption of the flour was determined as described by Bhatty (1986). The flour (4 g) was mixed thoroughly with 25 ml commercial soybean oil, in lieu of corn oil, using a mechanical stirrer. The mixture was allowed to stand at room temperature (33 ± 2°C) for 30 min and centrifuged at 1890 × **g** for 25 min. The free oil (supernatant fraction) was measured and expressed as percentage oil absorption. The height of dough with time during proofing was determined as described by He and Hoseney (1992) with slight modification. The moulded dough (50 g) was put into a 500 ml graduated glass cylinder. A very light (0.03 g) stick (4.5 × 0.1 cm) was placed on top of the dough and this acted as an indicator showing the change in height. The initial height of the dough before proofing was recorded using a scale. Then, dough was trans-ferred into a cupboard maintained at 30 ± 2°C and 75% relative humidity. Changes in dough heights were recorded at 15 min intervals for a streched period of 2 h. Loaf volume (ml/g) was estimated by dividing the loaf volume by its weight (g).

Sensory evaluation and statistical analysis : Coded samples of bread were presented to 10 panelists. They were instructed to score the following attributes-colour (crust and crumb), taste, flavour, texture and overall acceptability of the product using a 5-point Hedonic scale: 1, like extremely; 5, dislike extremely. Analysis of variance was carried out on the data (Steele and Torrie 1980).

The proximate composition data of flour and bread with and without shea fat are presented in Table 1. The bread with margarine served as control. The composition of flour compared fairly well with that reported by Yaseen et al (1991) for wheat flour except that there was no appreciable difference in composition between the two brands of bread. However, the moisture content of bread with shea fat was significantly ($P<0.05$) more than that with conventional shortening (margarine). This difference in moisture retention may be due to the relative high polar lipid content of shea fat (Badifu 1989). This may subsequently affect the stability of bread with shea fat.

Table 2 shows the fatty acid pattern of both the flour and shea fat. The predominant fatty acids were linoleic acid and stearic acid for flour and shea fat, respectively. The level of saturation of fatty acid was more in shea fat (53.6%) than in flour (24.9%). Consequently, oil from the flour was more unsaturated (75.1%) than shea fat (46.4%). The fatty acid patterns of the oil from flour compared fairly well with 21.5% palmitic, 1.3% stearic, 9.9% oleic, 58.6% linoleic and 4.5% linolenic as reported by Bhatty (1986). Those of shea fat were

TABLE 1. PROXIMATE COMPOSITION OF FLOUR AND BREAD (% DRY MATTER BASIS)

Parameter	Flour	Control*	Experimental**
Moisture, %	11.5 ± 1.5	21.1 ± 1.5	23.8 ± 1.7
Protein (N × 6.25)	9.7 ± 1.2	11.2 ± 0.7	10.5 ± 9.6
Fat, %	1.8 ± 0.6	1.5 ± 0.3	1.6 ± 0.3
Ash, %	1.3 ± 0.2	1.6 ± 0.5	1.8 ± 0.4
Fibre, %	1.1 ± 0.3	1.4 ± 0.3	1.5 ± 0.2
Carbohydrate (by difference)	76.6 ± 0.8	62.2 ± 1.0	60.8 ± 1.2

Means ± Standard deviation n = 3

* Containing margarine as shortening

** Containing shea fat as shortening

TABLE 2. FATTY ACID METHYL ESTER COMPOSITION (%) OF FLOUR AND SHEA FAT

Sample	16:0	18:0	18:1	18:2	18:3	Unsaturated	Saturated
Flour	23.5	1.40	10.6	58.8	5.7	75.1	24.9
Shea fat	5.3	48.3	40.1	6.0	0.3	46.4	53.6

n = 3

at variance with 1-3% linolenic acid reported by Badifu (1989) for shea fat phospholipids. These patterns explain, perhaps, why oil from shea kernel is always solid at ambient temperature (20-32°C). At solid state, the shea oil is called shea fat in which state, it is milky in appearance, whereas, when melted to liquid (oil) state, it is pale yellow in colour. This natural solid state characteristic has prompted its being tried as shortening in breadmaking. The oil absorption capacity of the flour was 66.9%, whereas its sugar content was 3.2%.

Table 3 presents data on the heights attained by dough with and without shea fat during proofing to a maximum period of 2 h. The initial height of both dough was 4 cm. A gradual increase in height with proofing was observed in both to a maximum height of about 13 cm in 45 min. There was no further appreci-able increase beyond 45 min. Statistical analysis showed no significant difference ($p>0.05$) in dough height between the dough with shea fat and the dough with margarine. Similarly, the loaf weight, volume and specific volume did not appreciably differ. Dough height depends, to some extent, on the volume of gas (CO_2) production during fermentation and proofing as well as the protein matrix. During baking, some doughs collapsed due to perhaps, escape of gas from their wall. This happens when wheat protein-gliadin (for loaf volume potential and gas retention) and glutenin (closely related to mixing tolerance and other important physical dough properties) fail in their function. Flour lipids are important in baking (Pomeranz et al. 1991). The lipid components of shea fat comprise 96% non-polar, 3% polar (phospholipids) and 1% glycolipids (Badifu 1989). Dafatting of wheat flour has been reported (Chung et al. 1980, 1982) to timpair loaf volume and grain of bread baked from the flour. They further reported that the addition of non-polar lipid damaged the quality of defatted flour, whereas the addition of polar lipids increased loaf volume and improved texture. These observations, perhaps

TABLE 3. DOUGH HEIGHT DURING PROOFING AT 32±2°C AND 75% RELATIVE HUMIDITY

Time, min	Dough height (cm) with Margarine	Shea fat
0	4.0	4.0
15	6.0	6.0
30	10.0	11.9
45	13.3	13.4
60	13.1	13.2
75	13.2	15.3
90	13.2	13.2
105	13.2	13.2
120	13.2	13.0

n = 3

TABLE 4. PHYSICAL AND SENSORY CHARACTERISTICS OF BREAD

Parameter	Bread Control*	Experimental**
Loaf weight, g	448	450
Loaf volume, ml	856	860
Specific volume, ml/g	1.91	1.91
Flavour	2.45[a]	2.50[a]
Colour	2.55[a]	2.70[b]
Taste	2.60[c]	2.67[c]
Texture	2.55[a]	2.68[d]
Overall acceptability	2.60[c]	2.66[c]

* containing margarine as shortening

** containing shea fat as shortening.

Means in the same row with different superscripts indicate significant difference ($p<0.05$) Scale 1 to 5 (1, like extremely; 5 dislike extremely)

explain the slight difference in loaf volume between the bread with shea fat and that with margarine (Table 4). The weight and specific volume of the loaves were comparable. Organoleptically, they were similar with uniform fine-textured crumb, flavour, taste, texture and smooth white surface. There was no significant difference ($p>0.05$) between the two loaves in terms of overall acceptability (Table 4). Therefore, it is suggested that crude shea fat could be used as a shortening in breadmaking and the qualities of the bread were comparable to those made with margarine.

Conclusion

The use of crude fat as shortening in breadmakging has been found to be quite feasible. The bread in which it was used as shortening demonstrated comparable qualities and enjoyed overall acceptability with bread containing conventional shortening such as margarine. The use of shea fat would further diversify its food values in Nigeria and other countries where shea tree is wildly grown in abundance. To meet the future demand for shea fat in Nigeria and other endowed countries because of its food and pharmacological potentials, there is the need to encourage the establishment of shea tree plantations. Further development of improved variety of shea tree is advocated.

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Osmotic Dehydration of Carrot Shreds for *Gazraila* Preparation

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Dehyration of carrot shreds was carried out by concentrating the material first in sucrose solution (50°Brix) at room temperature prior to drying in cabinet drier at 55 ± 1°C. Half of the initial moisture content was removed during the initial 30 min of osmosis and additional 6 h were required to reduce the moisture content of the osmosed carrot shreds to 5.8%. On the other hand, for the un-osmosed carrot shreds, the drying time was 12 h at 55 ± 1°C. The moisture sorption isotherm studies revealed that the un-osmosed dehydrated carrot shreds were more hygroscopic as compared to the osmosed dehydrated sample and required a lower RH for safe storage. *Gazraila* made from osmosed dehydrated shreds received higher scores for all sensory parameters.

Keywords : Carrot shreds, Dehydration, Osmosis, *Gazraila*.

Osmotic dehydration is an effective technique for the preservation of fruits and vegetables, realized by placing the solid food, whole or in pieces, in sugar or salt aqueous solution of high osmotic pressure. Concentrated sucrose solutions (50-70° Brix) are commonly used for concentration of carrot, apple, pineapple, amla etc. It gives rise to at least two major counter-current flows, water flowing out of the food into the solution and a simultaneous transfer of solute from the solution into the food (Lenart 1992).

The advantages of osmotic dehydration are its effectiveness at ambient temperature, better colour and flavour retention along with a reduced energy input over convective drying processes. But osmosed food products have to be further processed (Generally by air, vacuum or freeze-drying methods) to obtain a shelf-stable product. Hot-air-drying followed by osmotic dipping is commonly used (Ponting et al. 1996; Jackson and Mohamed 1971). Sucrose is the best osmotic agent because of its effectiveness, convenience and desirable flavour (Ponting et al. 1966).

Carrot is one of the most nutritious vegetables consumed in raw and processed form. *Gazraila* is a sweet dish prepared by cooking shredded carrot in milk and sugar and is much liked in North India. However, the product is not available throughout the year because of the fact that carrot is a seasonal crop of perishable nature. The shelf life of carrot can however, be increased by either osmotic or convective drying or a combination of both. Osmotic dehydration of large pieces of carrot has been studied (Choi and Youn 1995). However, no work has been reported in the literature on carrot shreds. The objective of this study was to study dehydration of carrot shreds both osmosed and un-osmosed and to see if the dehydrated product could be used for *gazraila* preparation.

Fresh carrot was procured from the local market, manually peeled, washed with potable water and placed on a sieve to drain out surface water. The peeled carrot was shredded to 8 mm × 3 mm × 1mm size, using a locally available commercial food processor. The shredded material was blanched for 2 min in hot water at 80° C, followed by cooling in cold water. The samples after draining of excess water were dipped in 50°Brix sugar syrup in the ratio of 4:1 (Rahman and Land 1990). A small portion (4-5 g) of the carrot shreds under osmosis was removed from the syrup wiped and analyzed for moisture content at regular intervals. The osmotic concentration of the shredded sample was continued for 2 h. The osmosed sample was separated from the syrup and immediately dried using hot air at 55 ± 1°C in a cabinet dryer (Narang and Co. New Delhi). The cabinet dryer was adjusted to the selected temperature, at least 0.5 h before the start of experiment. The samples of about 2 kg initial mass each were uniformly spread (2 kg/m²) on trays and subsequently placed in the drying chamber. The observations on the moisture loss were taken at regular intervals by withdrawing about 5 to 10 g sample for moisture determination. The samples were turned after every 0.5 h to achieve uniform drying. The drying was continued till the material was dried to approximately 5.6% moisture content (db). The dried samples were cooled to room temperature and immediately packed in polyethylene (250 gauge LDPE) bags for further analysis. Moisture content and the amount of sugar coated on the dehydrated osmosed carrot shreds were determined according to the method described by Ranganna (1986).

In another experiment, un-osmosed carrot shreds were dried to about 6% moisture content (db) by drying the blanched sample in a cabinet dryer as metntioned above. Equilibrium moisture content of the dehydrated samples was determined at 20, 40, 60, 80 and 100 % relative humidities at room temperature using the static method (Ruegg 1980). The desired relative humidity in the desiccator was maintained by using sulphuric acid solution of different concentrations. The samples were weighed every alternate day. The equilibrium was considered to have been achieved, when the change in two subsequent readings was less than 10 mg. In general, it took about 20 days for the sample to reach an equilibrium with the surrounding air.

During *gazraila* preparation, the ratio of carrot solids to sugar was kept at 1:0.8 and the ratio of carrot solids and sugar to milk was 1:2.2. The un-osmosed carrot shreds (100 g) were cooked with 80 g sugar and 400 ml of standardized milk (4.5 % fat and 8.5% solids-not-fat) in a heating pan to obtain *gazraila* with a moisture content of 45% (wb). The

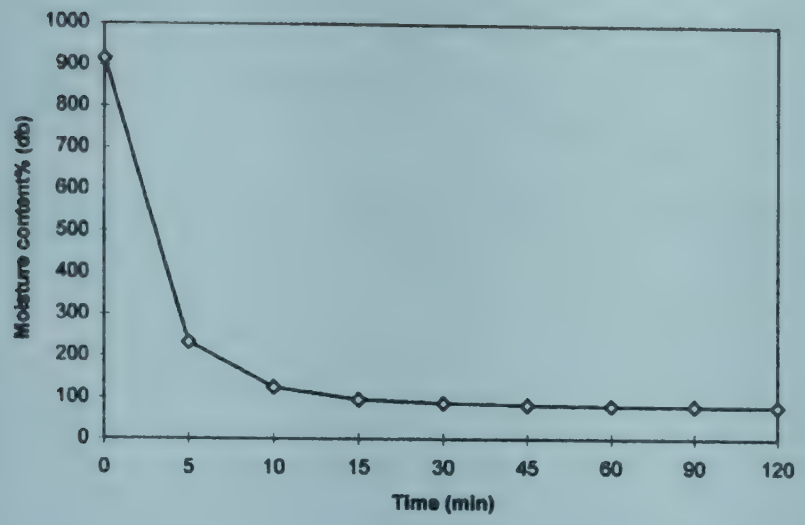

Fig. 1. Osmotic dehydration of carrot shreds in 50°B syrup at 25°C

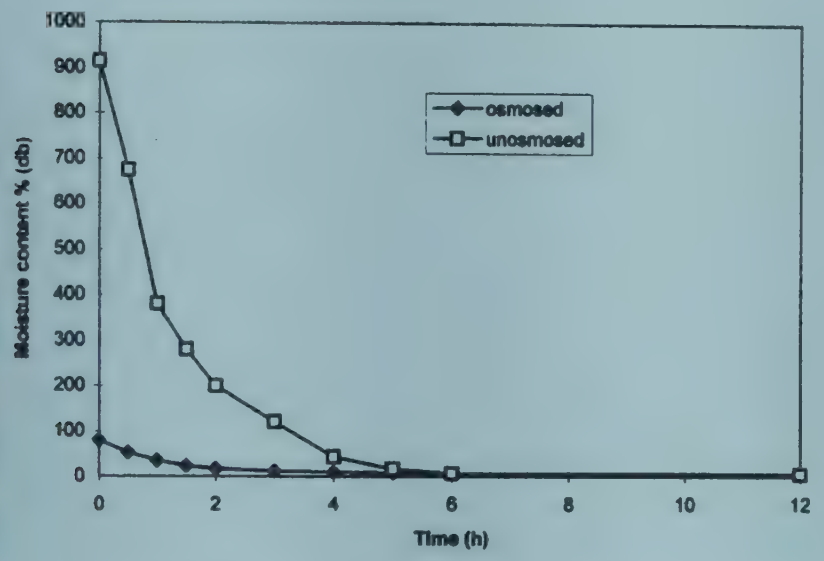

Fig. 2. Dehydration of osmosed and un-osmosed carrot shreds at 55°C

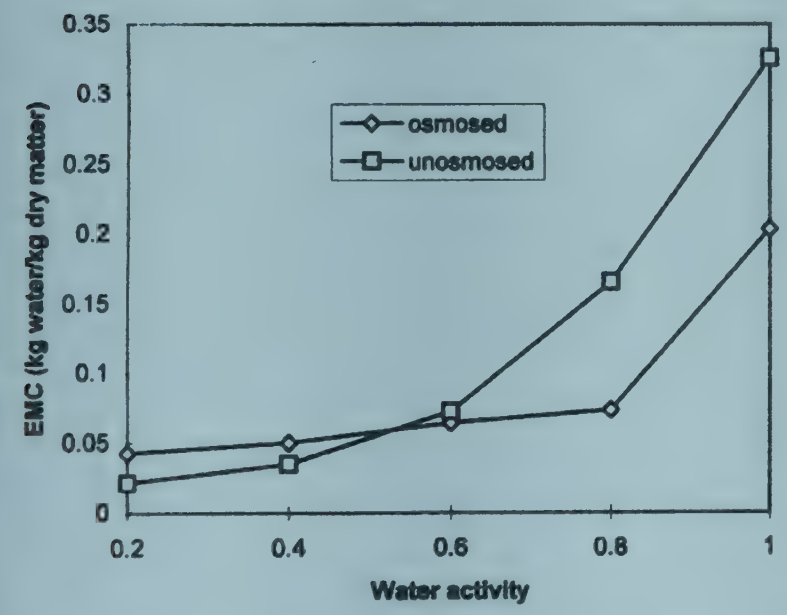

Fig. 3. Moisture sorption isotherm of osmosed and un-osmosed dehydrated carrot shreds

gazraila was prepared from the osmosed dehydrated carrot shreds similarly, by adding 220 ml of milk to 100 g of osmosed shreds without addition of the sugar. The sensory evaluation of the prepared *gazraila* samples was carried out by a semi-trained panel consisting of 20 judges for the overall acceptability, based on appearance, texture and flavour using a 9-point Hedonic scale. All the experiments were replicated thrice and the average values are reported.

TABLE 1. MEAN* SENSORY SCORE OF *GAZRAILA* PREPARED FROM OSMOSED AND UN-OSMOSED DRIED CARROT SHREDS

	Texture	Flavour	Appearance	Overall acceptability
Osmosed	8[a]	7.5[a]	8[a]	8.2[a]
Un-osmosed	7[b]	6.5[b]	5[b]	6.3[b]

* Means superscripts with the same letter in columns are not significantly different (P>0.05)

The yield after peeling and shredding the carrots was 75%. The sugar content of the osmosed dehydrated carrot shreds was 45.2%. Removal of moisture as a function of time when the carrot shreds were dipped in a sugar syrup of 50° Brix concentration is shown in Fig. 1. Almost 95% (db) moisture was removed from the carrot shreds during the initial 30 min of soaking. An equilibrium was reached after 2 h of soaking. Earlier, Conway et al (1983) and Giangiacomo et al (1987) reported that water loss during osmotic dehydration mainly occurred during the first 2 h of soaking. Fig. 2 shows the loss in moisture content during dehydration of the osmosed carrot shreds in a cabinet drier at 55 ± 1°C. The moisture content of the osmosed shreds was reduced from 80.18% (db) to 6.04% (db) in about 6 h. It required almost 12 h to dry the un-osmosed carrot shreds from 95.3% (db) to 5.82 % (db) (Fig. 2). It was, therefore, deduced that osmodehydration of carrot shreds led to a reduction in drying time by 6 h and thus a lower energy expenditure.

The sensory scores (Table 1) of the *gazraila* prepared from the osmosed and un-osmosed dehydrated carrot shreds showed that these were of acceptable quality. However, the *gazraila* prepared from osmosed dehydrated carrot shreds received higher score for appearance and overall acceptability. Further, the water absorption was more rapid in case of un-osmosed carrot shreds as compared to osmosed carrot shreds (Fig. 3). This clearly shows that the un-osmosed carrot shreds are more hygroscopic and will require a lower RH and special packaging for safe storage, whereas the osmosed carrot shreds are less hygroscopic and will have a better shelf life.

It was concluded from this study that osmotic dipping of carrot shreds before dehydration led to a substantial loss in moisture content and as a result, the drying time was reduced. The resulting product was less hygroscopic and had a better keeping quality and *gazraila* prepared from the dehydrated osmosed carrot shreds was found to be more acceptable.

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RESEARCH NOTE

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Identification of Gamma Irradiated Pulse Seed (*Lens* sp.) Based on Germination Test

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The germination test of pulse seed provided a reliable method for the identification of lentil seeds that had been subjected to irradiation. Root and shoot lengths were found more sensitive to the gamma irradiation than the germination percentages. The critical dose that prevented the root elongation varied from 0.1 kGy to 0.5 kGy. Germination percentage was reduced drastically above 0.2 kGy. Above 1.0 kGy dose, the lentil seeds did not germinate. The sensitivity of lentil seeds to gamma irradiation was inversely proportional to moisture content of the seeds. In addition, storage period up to 12 months had little effect on irradiation the induced reduction of root and shoot lengths. Thus, this test can determine the difference between irradiated and non-irradiated lentil seeds even 12 months after gamma irradiation.

Keywords : Germination test, Gamma irradiation, Lentil seeds, Root/shoot lengths.

Lentil (*Lens* sp.) is one of the most important pulse crops in the world, including India. Despite the availability of many food processing technologies developing countries are still experiencing high post-harvest losses of food. The crop seeds are easily infected with insects during storage. To combat this problem, cereals, pulses and other stored products are preserved by chemical fumigation. Their use has created problems relating to health and environment. Some countries have prohibited irradiation. But some countries that do permit food irradiation insist on appropriate labelling on foods, which have been subjected to irradiation (Bogl 1990; Delince and Ehlermann 1989). Detection methods are also required for enforcing good process control by regulating agencies (Ehlermann 1996). Only a few methods are available for a limited number of foods. Methods based on biological changes such as inhibition of seed germination and elongation of roots or shoots from germinating seeds have been reported for detection of irradiated cereal grains and legumes (Kawamura et al. 1992a, b; Qiongying et al. 1993; Zhu et al. 1993). The failure of produce roots was suggested as a sound method for identification of irradiated onions (Munzener 1976). Atsumi and Matano (1973) reported that the irradiation of wheat seeds reduces shoot and root lengths upon germination. Recently, Selvan and Thomas (1999) have evaluated the rooting characteristics and rate of root elongation in irradiated onions and shallots. However, no techniques are currently available for the identification of irradiated pulse seeds.

Therefore, a germination test for the identification of irradiated lentil seeds has been standardized. The effects of gamma irradiation, moisture contents in seeds and duration of storage after gamma irradiations have been studied and the results are reported in this communication.

The local cultivar of lentil seeds (*Lens* sp.) that had not been treated with insecticides were used in the present study. To study the effect of storage time on germination, non-irradiated lentil seeds were stored at -20°C and at room temperature prior to germination.

Lentil sedds were gamma-irradiated with doses of 0.1, 0.2, 0.5, 1.0 and 2.0 kGy in a GC-9000 ^{60}Co gamma irradiator. Dose rate was 0.452 kGy/h. Absorbed doses were determined using Fricke Dosimeter. After treatment, the samples were tested within two days. In the studies of the storage after irradiation, the samples were stored at room temperature prior to germination.

The effect of moisture content on radio-sensitivity to gamma irradiation was studied by changing the moisture content of lentil seeds, by storage with silica gel for one month. Moisture content was established by comminuting the seeds and determining the loss on drying at 105°C.

Each germination test was carried out with at least 50 seeds in a set and each set was repeated at least three times. The seeds were imbibed for 10 h in distilled water and placed on distilled water-moistened absorbent cotton layer of 9 cm cover petri dish and cultured at 28°C in plant growth chamber. Germination percentages as well as root and shoot growth in terms of lengths were measured. Protuberance of radicals was taken as the initiation of germination. The germination percentage, shoot and root lengths were noted after 5 days of initiation of germination (Banerjee 1998). Shooting and rooting were defined as protrusion of the shoot and root to the extent of at least 0.5 mm.

The parameter, 50% Inhibition Dose Root (IDR_{50}), was used as a measure of radiosensitivity. IDR_{50} is the amount of irradiation that reduces root length of 50% of that of non-irradiated pulse seeds. It was determined by plotting irradiated dose versus the root length achieved after 5 days of culturing.

The temperature of culture was studied in plant growth chamber and root and shoot growth proceeded most rapidly at 28°C was for the first 5 days. In the interest of establishing a quick test for evidence of irradiation, 28°C was adopted as culture temperature for the germination test. Previous storage for up to six months of non-irradiated pulse seeds at -20°C and at room temperature had no effect on germination, although there were some variations in shoot and root lengths (Table 1).

The effect of gamma irradiation on lentil seeds was studied. Seeds were irradiated with 0.1, 0.2, 0.5, 1.0 and

TABLE 1. EFFECT OF STORAGE ON ROOT AND SHOOT LENGTH OF LENTIL SEEDS AT ROOM TEMPERATURE AND -20°C. ROOT AND SHOOT LENGTHS SHOW THE MEANS OF ±SE AT 6 MONTHS OF CULTURE

Storage period, months	Root length, mm		Shoot length, mm	
	Room temp.	-20°C	Room temp.	-20°C
1	46	39	18	14
2	42	38	20	18
3	39	31	23	16
4	52	46	24	14
5	56	42	22	12
6	45	28	19	10

2.0 kGy. The seeds were imbibed and cultured for 5 days. Plate 1 shows the typical growth of gamma irradiated lentil seeds, where the lengths of roots were bigger than shoots in every case. Fig 1 shows the growth curves of lentil seeds at different doses. In 1.0 and 2.0 kGy sets, the rooting and shooting per-centages were more or less nil whereas, in 01, 0.2 and 0.5 kGy sets, rooting and shooting percentages were 89:85, 52:48 and 32:30 respectively. Higher doses inhibited germination. For this reason, only 0.1 to 0.5 kGy doses were used for the consideration of germination test. Germination percentages showed differences in response to different dose rates. In 0.5 kGy set, the germination percentage was very less as compared to control and 0.1 kGy sets. It indicated that

Plate 1. Typical growth of roots and shoots of gamma irradiated lentil seeds in different doses, after 5 days culture in plant growth chamber

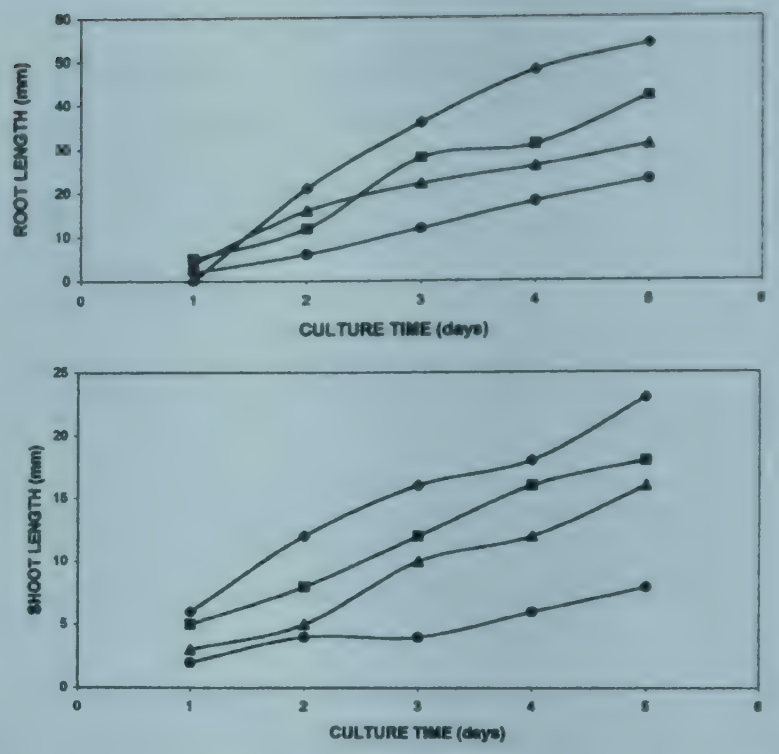

Fig. 1. Effect of gamma irradiation on growth of root and shoot in different doses. Non-irradiation (◆); 0.1 kGy (■); 0.2 kGy (▲) and 0.5 kGy (●). Root and shoot lengths show the mean ± SE

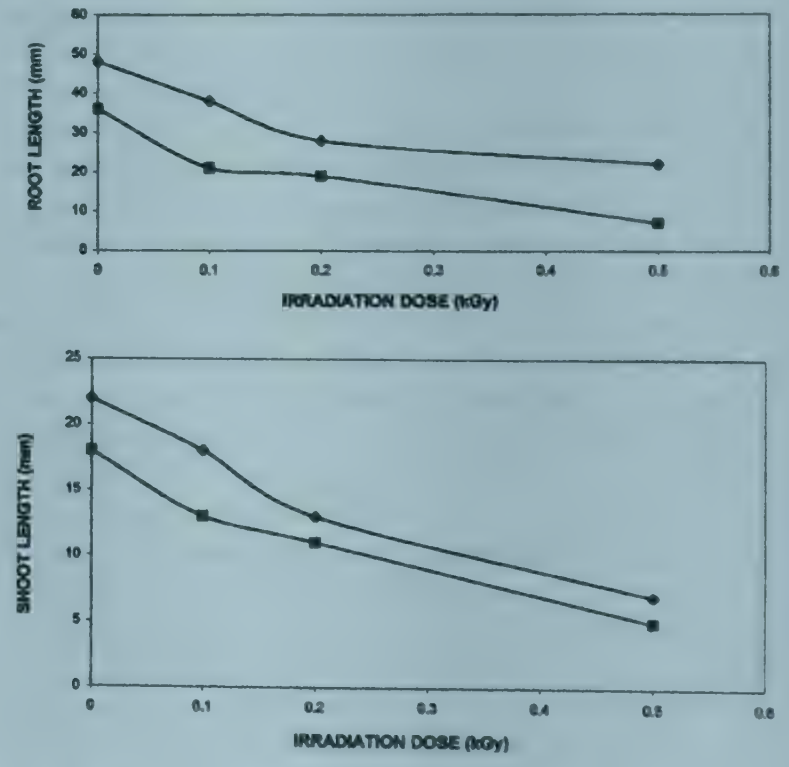

Fig. 2. Effect of moisture content (MC) on the root and shoot lengths reduction of lentil seed caused by gamma irradiation: control, MC 10.3% (◆); with silica gel MC 7.2% (■). Root and shoot lengths show the mean ± SE

in higher dose, germination percentage also showed difference in addition to root and shoot lengths. In lower dose i.e. 0.1. kGy, the germination percentage was not significantly differerent from control but root and shoot lengths were reduced markedly (Plate 1). Gamma irradiation in excess of a critical dose caused depression on shoot and root growth. The reductions of both shoot and root lengths are dependent upon the irradiation dose (Plate 1). The critical doses that significantly inhibited the 5 days root/shoot elongation ($P<0.05$) were from

0.1 to 0.5 kGy. Irradiation at 1.0 kGy caused depression of germination. There was no differences in germination percentages between non-irradiated and irradiated lentil seeds if irradiation dose was not above 0.1 kGy.

Ramarathnamm et al (1987) also reported that iradiation of rice seeds at level in excess of 5 kGy did reduce germination. The present observation also indicated that the shoot and root growth was more sensitive than germination percentages to gamma irradiation.

The effect of moisture content on radio sensitivity of pulse seeds to gamma irradiation was studied. To confirm the concentration, the moisture content of lentil seeds was reduced to 7.2% by storage with silica gel for one month. Lentil seeds stored under room temperature contained 10.3% moisture. These samples were gamma-irradiated and their germination behaviours were observed. The root and shoot lengths of lentil seeds dried with silica gel were reduced more remarkably by gamma irradiation (Fig. 2). This indicated that the sensitivity of lentil seeds to gamma irradiation was inversely proportional to moisture content. The low moisture content in the seeds was more sensitive to gamma irradiation and more moisture in seeds had higher resistance to gamma irradiation.

Long term storage (up to 12 months) of lentil seeds after gamma irradiation had little effect on the reduction of root and shoot lengths, which were irradiated with doses between 0.1 kGy and 0.5 kGy. It is important to note that there was growth related damage due to gamma irradiation up to 12 months of storage. Effect of irradiation can be detected even after one year of gamma ray exposure.

The variability as measured by standard deviation of the root/shoot lengths decreased with increase in the radiation dose. When irradiation was adequate to reduce the rooting percentages, the root lengths did not exceed a few millimeters in length. It has been speculated that free radicals generated in irradiated seeds might be related to growth inhibition (Kumagai et al. 2000). However, it is interesting that in lentil seeds below 0.1 kGy radiation dose did not show any effect on germination.

Germination test, based on root/shoot elongation, is an easy, rapid and reliable method, which can be used for the identification of pulse seeds that have been irradiated at a level in excess of 0.1 kGy, even if the seeds are stored for 12 months after irradiation.

The author is thankful to Director, Defence Laboratory, Jodhpur for providing infrastructural facilities for conducting this work. Gamma irradiation of seeds was performed in the gamma chamber of Rakhsa Anusandhan and Vikash Irradiator (RAVI)

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Development of a Cottage Industry for Dehydrating Whole Egg

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Whole egg fluid was heated in a small cabinet dryer. It was kept in petri dishes, which were lined with silver foil. The dishes were kept in the cabinet dryer. Heated air was supplied from a heat convector. Trials revealed that air temperature of 45-50°C for 1 mm thick layer of egg fluid and wind velocity of 92 mt/sec could dry the fluid in 6 h. It was further revealed that the drying reduced by 35 min, when whole egg fluid was kept in stainless steel trays line with aluminium foil. The flakes obtained after dehydration were ground to powder in a grinder. Omelette was prepared from a commercially available egg powder and that prepared from fresh egg. The comparison revealed that the overall acceptability of the omelette made from the egg powder prepared in laboratory was the second best.

Keywords : Omelette, Flakes, Granules, Foaming.

Production of eggs has reached an unprecedented peak, requiring development of a gigantic marketing network and processing industries. However, in India, eggs are still consumed fresh as omelette and boiled at kitchen level only. Also, most of the bakeries use it as a emulsifying agent in the preparation of cakes. Egg processing industry has only recently made its presence felt and a few plants have been commissioned. However, due to higher technical skill required and exhorbitant cost of the finished product, it is out of the reach of the common man (Parihar et al. 1997). The constraints are multi-directional. The huge egg processing plants complain of non-availability of raw materials, while poultry farmers complain that they do not get the suitable price for their products due to marketable surplus. At present, the manufacturing units are located in big cities, where the eggs are brought from egg producing areas. As such, they are likely to be subjected to shocks and jerks and get broken. The losses are estimated at 2 %. Development of cottage industries in production areas will reduce the percentage of brokens and generate employment for the local population.

The present study was planned with the following objectives. i) to develop a small hot air dryer for drying whole egg fluid. ii) to optimize drying parameters with respect to colour. iii) to evaluate the sensory attributes of the prepared egg powder and v) to test the techno-economic feasibility of the developed technology.

Dehydration of egg : Whole egg, egg white and yolk were dehydrated in a small cabinet dryer. Small petridishes were sterilized and lined with aluminium foil and filled with egg fluid to a depth of 0.25 to 1.00 mm. These dishes were kept in a cabinet and hot air was blown over the petri dishes. The cabinet dryer made of GI sheet of 350 mm width and 290 mm length is shown in Fig.1. It had a height of 95 mm. It was divided into three compartments in which dryer could be slided. These dryers were made of stainless steel and were perforated. A small chimney having 200 mm height, 40 mm diameter was provided on the last compartment to blow off the waste air from the cabinet. This cabinet was fixed to a heat convector (Usha Make) from where the hot air was blown. The experiment was conducted in petri dishes as mentioned earlier and also in perforated steel trays lined with aluminium foil. Various time temperature combinations were tried in the preliminary trials (Table 1) and best results was obtained at a temperature range of 45°C-50°C with an air velocity of 0.92 m/s and dehydration time of 6 h depending upon the thickness of egg fluid. The egg fluid after dehydration got converted into flakes and granules, which were ground to powder.

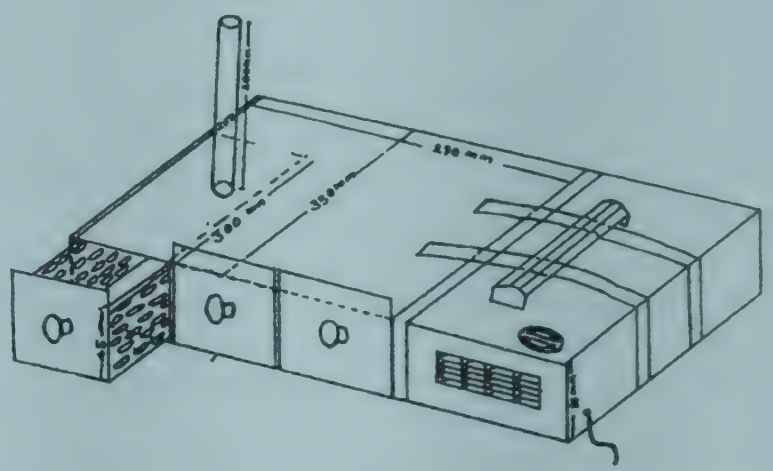

Fig. 1. Cabinet dryer developed in laboratory

TABLE 1. DEHYDRATION OF EGG TO 8% MOISTURE CONTENT BY VARIOUS PROCESS PARAMETERS IN THE DRYING CABINET

Item	Thickness of liquid, mm	Wind velocity, mt/sec.	Time taken for drying, h
Whole egg in petri dish	0.25	0.92	3.50
	0.50	0.92	4.15
	0.75	0.92	5.10
	1.00	0.92	6.00
Whole egg in perforated steel tray lind with aluminium foil.	0.25	0.92	3.15
	0.50	0.92	4.00
	0.75	0.92	5.25
	1.00	0.92	6.25

Determination of free fatty acids : Free fatty acids in the samples were determined at the beginning and end of experiment according to the method of Harold (1971).

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TABLE 2. EFFECT OF DEHYDRATING EGG ON FREE FATTY ACID CONTENTS

Item	Free fatty acids, %
Fresh	1.50
Dehydrated egg powder in laboratory	1.75
Market sample	2.10

TABLE 3. EFFECT OF METHOD OF PREPARATION OF EGG POWDER ON FOAMING PROPERTIES

Item	Initial volume, ml	Total volume after beating, ml	Rise in volume, ml	Percentage rise in volume, ml
Fresh egg	50.00	80.00	30.00	60.00
Fresh egg	50.00	78.00	28.00	56.00
Fresh egg	50.00	77.00	29.00	58.00
Fresh egg	50.00	80.00	30.00	60.00
Egg powder	50.00	55.00	5.00	10.00
prepared in Lab.	50.00	54.00	4.50	9.00
"	50.00	54.00	4.00	8.00
"	50.00	55.00	5.00	10.00
Market egg powder	50.00	52.00	2.0	4.00
"	50.00	51.50	1.5	3.00
"	51.80	51.80	1.8	3.60
"	50.00	52.00	2.8	4.00

TABLE 4. EFFECT OF DEHYDRATION OF EGG POWDER ON SENSORY ATTRIBUTES WHEN PREPARED AS OMELETTE

Item	Colour	Taste	Flavour	Texture	Total	Average value
Fresh egg	8.00	8.25	7.50	8.25	33.00	8.25
Egg powder prepared in laboratory	8.00	7.00	7.75	7.00	39.75	7.43
Market egg powder	5.00	6.50	5.75	5.50	22.75	5.68
Total	21.00	21.75	21.00	20.75	85.50	-
C.D. 5%	0.134					

Determination of foaming property : A mixture of 5 g of egg powder and 50 ml of distilled water was prepared and its volume was noted in a graduated cylinder. The mixture was then stirred for 2 min. The percentage increase in volume was then determined by the following expression.

$$\frac{\text{Final volume (ml)-Initial volume (ml)}}{\text{Initial volume}} \times 100$$

Determinination of sensory attributes : Omelette prepared from egg powder of various sources were subjected to sensory evaluation as per standard method (BIS-6273 Part I, 1972).

Moisture content : Moisture content of egg powder was determined by AOAC (1965) method.

Effect of drying parameters on free fatty acids : The values of free fatty acids presented in Table 2 indicate that the egg was bound to become rancid on dehydration. However, by dehydrating it with a less severe heat treatment, the rancidity was comparatively reduced. It was 1.75% by dehydration of fresh egg liquid in laboratory, whereas it was 2.10% in the sample procured from the market.

Effect of drying parameters on foaming property : The foaming properties were determined as per cent increase in volume. It can be seen from Table 3 that the fresh egg had the highest foaming property of 59%, followed by egg powder prepared in laboratory, which had foaming property of 9.2%. The egg powder procured from market had the minimum foaming property of 3.6%. This may be because of the heat treatment given to the sample during spray drying.

Sensory evaluation of cabinet-dehydrated egg powder : In order to study the effect of dehydration parameters on sensory properties of egg powder prepared in the laboratory, omelette were prepared from three different sources viz. from fresh egg, dehydrated egg powder prepared in the laboratory by the best treatment and egg powder procured from the market. A panel of 6 members was offered the omelette alongwith the score chart (Table 4). Each parameter was allotted 10 marks. The score cards were taken from the panel members after they had documented their opinion.

It is evident from Table 4 that colour and flavour of the omelette made from the egg powder prepared in the laboratory was at par with omelette prepared from fresh egg and much better than the omelette prepared from egg powder procured

TABLE 5. VARIATION IN RELATIVE HUMIDITY IN DIFFERENT PACKING MATERIALS AND ATMOSPHERE (4TH MARCH 1997 TO 24 MARCH 1997)

Packing treatment	Relative humidity in various packages, %				Average atmospher relative humidity, %			
	After 0 day (4th March 97)	After 7 days (11th March 97)	After 14 days (18 March 97)	After 21 days (24 March 97)	After 0 day (4th March 97)	After 7 days (11th March 97)	After 14 days (18 March 97)	After 21 days (24 March 97)
Thick polyethylene bag (150 guage)	40	45	37	42	-	-	-	-
Thin polyethylene bag (50 guage)	40	45	39	48	51.5	46.5	48.5	32.0
Coloured polyethylene (50 guage)	42	45	40	48	-	-	-	-
Aluminimum foil	42	42	42	45	-	-	-	-

TABLE 6. COST OF PREPARING 4 KILOGRAMS OF EGG POWDER PER DAY

Item	Amount, Rs.
Cost of equipments	10,000.00
Cost of egg to be dehydrated per day i.e. 400 eggs	600.00
Cost of electricity consumed per day 15 units at the rate of Rs. 2.5 for 2 shifts 37 × 2	75.00
Skilled labour charges (One labour/day)	90.00
Depreciation per day at 10% per year	3.30
Repairs and maintenance per day at per year	1.70
Housing charge per day 2% year	0.70
Bank interest per day at 15% year	4.10
Packing cost at 10% of the cost of product	76.00
Total (2 to 9) =	850.00
Amount of egg powder obtained per day in two shifts	4.4 kg
Cost of egg powder at market rate at Rs. 380/kg	1,672.00
Thus , profit/day from two shifts	822.00

from the market. However, taste and texture of omelette prepared in the laboratory was significantly inferior than the omelette of fresh egg and significantly superior than the omelette prepared from the market samples (Critical difference = 0.134). Overall sensory attributes of omelette prepared from the fresh egg were significantly better than the omelettes prepared from other two sources. Further, omelette made from laboratory dehydrated egg powder was also significantly superior than that from the egg powder procured from market.

It is seen from Table 5 was that for the first seven days, the packaging material was not much instrumental in the variation of the relative humidity inside it. However, on the fourteenth day, there was difference in relative humidity. This may be due to the fact that various package materials had different water vapour permeabilities. Therefore, the RH in thin polyethylene and coloured polyethylene on the 21st day was high (48%) as these materials have low permeabilities and atomospheric water vapour had gone inside the package material, thereby increasing the relative humidity. The variation of relative humidity in packages was proportional to the variation of atmospheric relative humidity, thus indicating that all the package materials which were considered were not 100 % impermeable.

The economics of the cost of production of one kilogram of egg powder are given in Table 6. A close look at the Table reveals that by dehydrating and grinding 4.5 kg of egg powder per day, a net profit Rs. 822/day can be made. Thus, it may be concluded that the technology developed is economically feasibile at cottage level.

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RESEARCH NOTE

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Studies on Microfiltration as a Method of De-lipidization of Whey for Production of Whey Protein Concentrate

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Cross-flow microfiltration (MF) of whey was carried out as a de-lipidization treatment prior to ultrafiltration (UF) for pilot-scale production of whey protein concentrate (WPC). Under isobaric MF conditions, the initial permeate flux was higher at a higher pressure (1.5 bar), but it declined sharply and in effect, reached a lower equilibrium value than that at the lower pressure (1.0 bar), where an almost uniform flux (mean, 74.3 lm^{-2} h^{-1} as compared to 75.8 lm^{-2} h^{-1} at 1.5 bar) was observed. When the pressure was increased stepwise during the run from initial 1.0 bar to 1.5 bar and at a later stage to 2.0 bar, the flux increased sharply at each stage. Thermocalcic pre-treatment of whey (0.025 Mol $CaCl_2$, 55°C for 8 min) marginally improved flux during MF, the average values being 74.5 and 79.8 lm^{-2} h^{-1} for untreated and treated whey, respectively. The protein permeation generally decreased as the MF run progressed, but it was somewhat higher for treated (54.9%) than that of untreated whey (48.4%). Without the pre-treatment of whey, protein permeation was slightly higher (50.5%) at 1.5 bar than that at 1.3 bar (48.4%). The effect of MF of whey on the permeate flux during subsequent UF was studied as a function of time. Though the initial flux for micro-filtered whey was higher than that for non-microfiltered whey, the final equilibrium flux was lower. Compositional analysis indicated a definite reduction (72.1%) in the fat content of WPC as a result of MF de-lipidization.

Keywords : Microfiltration, Ultrafiltration, Permeate flux, Transmembrane pressure, Whey protein concentrate, De-lipidization.

Whey, the fluid obtained, during cheese making, upon separation of the coagulum from whole milk, skim milk or cream contains milk solids (6 to 7%). Disposal of untreated whey would pollute the environment. Moreover, research has unequivocally established the excellent nutritional and functional properties of whey solids, especially the whey proteins (Kennedy 1985; Sienkiewicz and Riedel 1990; Jelen 1991; Patel et al. 1991). Recovery of whey solids in the form of food ingredients has considerably helped in solving the pollution problem, while at the same time providing high quality whey proteins as functional ingredients in human foods.

Production of whey protein concentrate (WPC) has been found to be a novel proposition for the whey industry (Pepper and Pain 1987; Marshall and Harper 1988; Jayaprakash et al. 1995). Commercial WPC is prepared by ultrafiltration (UF) of cheese whey and the product has a range of compositions depending on the degree of UF concentration achieved. The UF process has been found to reach limiting fluxes too soon despite clarification of the whey. This results in reduced efficiency of the system and higher cleaning costs. In addition, commercial WPCs are found to have varying compositions, which lead to irregular functional properties in the food formulations (Morr and Ha 1991; Daufin et al. 1993, Gesan et al. 1993, Karleskind et al. 1995). Moreover, the fat content in WPC, which ranges from 3 to 7% is known to impair its functionality, especially the whipping properties. Also, unsaturated fatty acids present in the milk fat are susceptible to oxidation and thus contribute to off-flavour development in the WPC, which is best prevented by complete removal of the milk fat prior to its concentration (McDonough et al. 1974; De Boer et al. 1977). However, conventional centrifuge technology is not capable of removing the smaller fat globules and phospholipids present in whey.

Microfiltration (MF) of sweet cheese whey has been shown to be a potential solution to the fat removal problem (Merin et al. 1983; Anon 1999; Lio et al. 1999). Several studies have since been carried out to optimize the process of micro-filtration for the whey industry (Pearce et al. 1991a, Gesan et al. 1993; Surel and Famelart 1995; Sharma 1996). De-lipidized whey protein concentrates have been successfully prepared by groups of researchers in various parts of the world. However, no effort has been made to determine the validity of the reported results under Indian conditions. In the light of the prevailing scenario, an attempt was made to optimize the processing parameters for MF of whey and the manufacture of de-lipidized WPC. The results are discussed in this communication.

Cheddar cheese whey : Cow/mixed milk cheddar cheese whey (in 200-300 lots) was collected from the Experimental Dairy of National Dairy Research Institute, Karnal. The cheese whey was clarified using a cream separator (500 l/h) and then heated (75°C/15s) in a plate heat exchanger (Silkeborg, Sweden). Its pH was adjusted to 6.5 by addition of 0.1N NaOH or HCl.

Ultrafiltration of whey : Cheddar cheese whey was subjected to ultrafiltration for the production of WPC by using a pilot-scale of hollow fibre membrane unit (Romicon, membrane type PM-50; 50,000 mol. cut-off, effective membrane area 2.54 m^2), supplied by Alfa-Laval, Sweden. The whey was con-centrated to a volume concentration ratio of up to 10. UF flux was determined by measuring the amount of permeate coming out of the membrane in a given time for the given area of the membrane and expressed as $lm^{-2}h^{-1}$. UF volume concentration ratio (VCR) was calculated by using the following formula :

$$VCR = \frac{V_o}{V_R}$$

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where V_o = Original volume of whey in litres

V_R = Volume of retentate, in litres

De-lipidization of whey by microfiltration : Clarified, pasteurized whey was subjected of MF using a pilot plant (Carbosep membrane, 0.2 μ,0.16 m^2 area), supplied by Tech-Sep (Miribel, Cedex, France). The processing conditions used were, temperature of whey 50°C, pH of whey 6.5, inlet pressure 1.6 bar, outlet pressure 1.0 bar and volume concentration ratio 4.0. During MF of whey, the flux was recorded at regular intervals of VCR. After 4 - fold concentration, the MF permeate was ultrafiltered under the same conditions as described above. In order to enhance the performance of the MF process, thermocalcic pre-treatment of whey (Fauquant et al. 1985) was used. It involved addition of calcium chloride (2.0 M solution) to whey at the rate of 1.0 g Ca^{2+} per litre (i.e. 0.025 Mol), pH was adjusted to 7.3 using 1 N NaOH solution, heated to 55°C and held for 8 min before MF at 50°C.

Protein permeation during MF (i.e. that fraction of the total proteins of initial whey, which passed into the MF permeate) was calculated using the following formula :

$$\text{Per cent protein permeation} = \frac{C_p}{C_w} \times 100$$

where C_p = Protein content in composite permeate (g)

C_w = Protein content in initial whey (g)

Spray drying of WPC : The WPC obtained as UF retentate was dried by using an Anhydro (Denmark) spray dryer (capacity, 35 kg water evaporation per hour). The inlet and outlet air temperatures were 180°C and 85°C, respectively.

Analytical methods : Total solids content was determined by gravimetric method (ISI, 1981). Protein content of whey protein concentrate was estimated by the Kjeldahl method using the Kjeltec apparatus (Tecator Digestion System 12 100 g Digestor and 1026 Distillation Unit) and taking 2 to 3 g sample in case of liquid products and 0.2 to 0.3 g sample in case of dried products for digestion with a single Kjeltab. Distillation was carried out in the distillation unit selecting the programme for appropriate levels of alkali. Fat content was determined by Mojonnier method given in the Laboratory Manual (1959), ash content by the AOAC (1984) method and lactose content was calculated by difference.

Effect of operating pressure on permeate flux during microfiltration : It can be seen from Fig.1 that the flux declined with increasing process time but the decline was of much smaller order (from initial 84.5 $lm^{-2}h^{-1}$ to 69.0 $lm^{-2}h^{-1}$ after 2.5 h) at 1.0 bar as compared to that at 1.5 bar (from initial 112.5 $lm^{-2}h^{-1}$ to 66.0 $lm^{-2}h^{-1}$ after the same process time). The decline in flux was rapid during the first hour of operation at 1.5 bar, but slow at the lower pressure. The flux after this initial period nearly stabilized the equilibrium value being somewhat smaller at the higher pressure. The relatively rapid decline of flux during the early MF run may be attributed to the pressure-governed fouling of the membrane, which is known to reach a limiting value after which, there is little further drop in the flux (Merin et al. 1983).

When the pressure was increased after 75 min of operation, from 1.0 to 1.5 bar, the flux correspondingly increased from 71.3 to 100.5 $lm^{-2}h^{-1}$, which subsequently declined during the next 45 min (Fig. 1). Similarly, when the pressure was again increased at a later stage from 1.5 to 2.0 bar, the permeate flux went up from 82.5 to 111.0 $lm^{-2}h^{-1}$, followed by a steady decline during the subsequent run. The decline in flux at each of the two stages was, however,not that rapid, when a constant pressure of 1.5 bar was used from the beginning, with the result that the mean flux was 75.8 $lm^{-2}h^{-1}$ for a 2.5 h run at 1.5 bar, whereas it was 85.5 $lm^{-2}h^{-1}$, when the pressure was increased in two stages (mean pressure, 1.5 bar). This may be attributed to a presumably dense fouling layer forming, when uniform higher pressure was used during the run, unlike when the process began at a low pressure, permitting the formation of a relatively loose fouling layer, which would be less prone to compaction upon subsequent increase in pressure during the run. Microfiltration of whey at pressures similar to those used in the present study has been reported to exhibit comparable permeate flux (Daufin et al. 1993).

Effect of thermocalcic pre-treatment of whey on microfiltration permeate flux : It is evident from Fig. 2 that the pre-treatment marginally improved the flux during MF, the average values being 74.5 and 79.8 $lm^{-2}h^{-1}$ for untreated and treated whey, respectively. Pre-treatment of whey with calcium chloride has been reported to result in the aggregation of lipoproteins which, in turn, are believed to improve the permeate flux presumably through formation of less dense fouling layer on the membrane. However, since the increase in flux after thermocalcic treatment was rather small in the present study, the pre-treatment did not show any practical significance.

Protein permeation during microfiltration : Table 1 shows that as MF progressed, the protein content in the permeate declined steadily. However, the quantity of protein recovered in the composite permeate was slightly higher, when MF was carried out at 1.5 bar as against at 1.3 bar. It is further seen from Table 1 that the protein content of permeate decreased with increasing concentration ratio in the case of untreated whey, but no definite pattern was observed in pre-treated whey, although the protein content of composite whey was higher in the latter. The pre-treatment of whey correspondingly resulted in a slightly improved protein recovery, which was in agreement with that reported by Gesan et al (1993).

Effect of microfiltration of whey on UF permeate flux : The permeate flux during UF of microfiltered and non-microfiltered whey as a function of process time is shown in Fig. 3. The initial flux was appreciably higher in case of microfiltered whey. However, it declined much more rapidly during the first two hours and the final flux was perceptibly lower than that in the case of untreated whey. Consequently, the mean flux was the same (11.7 $lm^{-2}h^{-1}$) in both the treated and untreated wheys. Merin et al (1983) reported a significantly improved flux during ultrafiltration of sweet cheese whey, which had been microfiltered with a 1.2 μm membrane. They attributed the improved UF permeate flux when using microfiltered whey to the absence of fat globules and casein

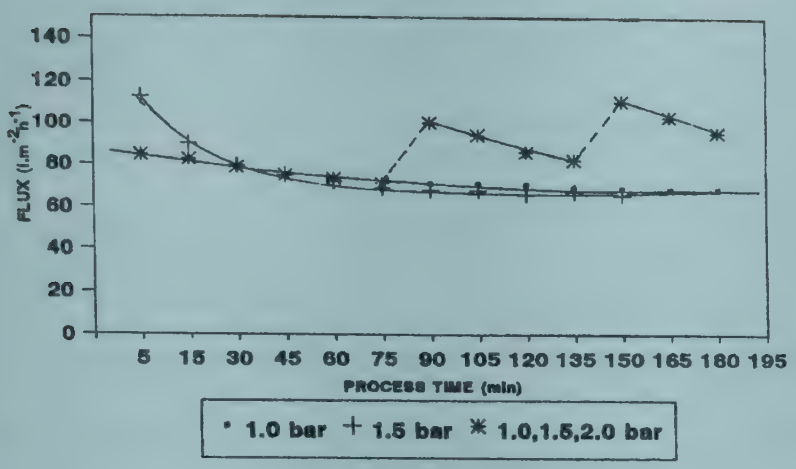

Fig. 1. Effect of transmembrane pressure on flux during microfiltration of whey

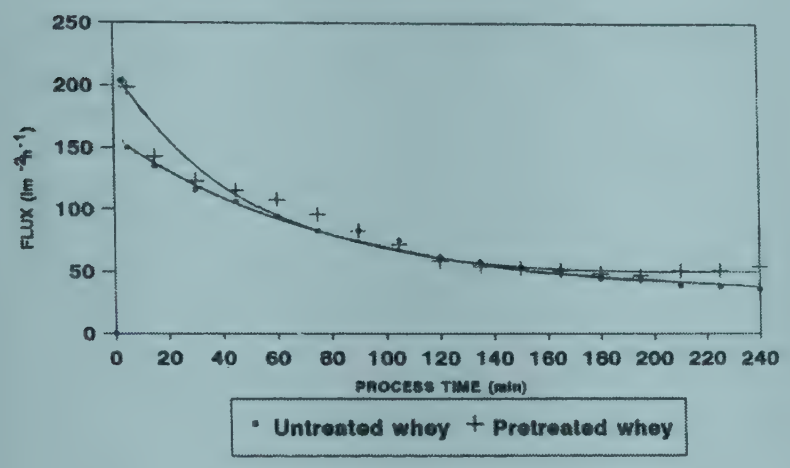

Fig. 2. Effect of thermocalcic pre-treatment of whey on flux during microfiltration (1.3 bar)

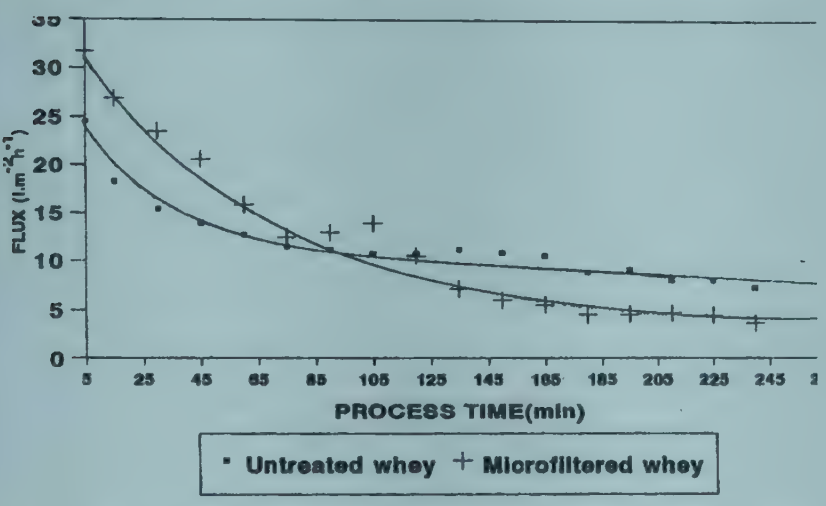

Fig. 3. Effect of microfiltration (1.30 bar) of whey on flux during subsequent ultrafiltration (1.15 bar)

fines in the feed stream, as these particulate entities are believed to be the major contributors to fouling of the UF membrane.

Effect of microfiltration of whey on composition of WPC: The de-lipidized WPC had a considerably reduced (by 72.1%) fat content (1.70-1.72%) as compared to the undelipidized product (5.97-6.29%). The protein (25.62-43.37%) and lactose (40.44-61.96%) contents were generally dependent on the degree of concentration, irrespective of the MF treatment. An increase in VCR, increased the protein content and correspondingly decreased the lactose content. The ash

TABLE 1. EFFECT OF TRANSMEMBRANE PRESSURE AND THERMOCALCIC PRE-TREATMENT OF WHEY ON PROTEIN PERMEATION (% PROTEIN IN PERMEATE) DURING MICROFILTRATION

Permeate sample drawn at	Untreated whey Pressure, 1.3 bar	Untreated whey Pressure, 1.5 bar	Treated whey Pressure, 1.3 bar
VCR, 1.0	0.46	-	0.43
VCR, 2.0	0.36	0.52	0.41
VCR, 3.0	0.33	0.50	0.52
Composite permeate	0.40	0.53	0.44
Protein permeation, %	48.4	50.5	54.9

Protein content of initial whey: being 0.66 % (1.3 bar experiments) or 0.86 % (1.5 bar)

contents of the two types of WPC (5.22-6.94%) did not show any definite trend. Considerable reduction in the fat content of the WPC has been reported by other workers, employing microfiltration as a pre-treatment (Pearce et al. 1991a, b). Pearce et al. (1991a) observed a fat reduction of 30 to 80%, depending on the type of MF membrane used, a 1.4 µm membrane giving lower reduction than 0.8 µm one.

In conclusion, microfiltration could be effectively employed as a pre-treatment of sweet cheese whey for de-lipidization aimed at production of a whey protein concentrate with reduced fat content. However, the transmembrane pressure conditions would appreciably affect the performance of the MF process in terms of the permeate flux. Intermittent (stepwise) increase in the pressure appeared to improve the overall flux. Thermocalcic treatment of whey prior to MF was only slightly effective in improving the flux. Microfiltration as a pre-treatment of whey could perceivably decrease the fat content of the whey protein concentrate obtained by subsequent ultrafiltration of the MF permeate.

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RESEARCH NOTE

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Microbiological and Biochemical Changes During Fermentation of *Kanji*

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A fermented beverage *kanji* was prepared from crimson coloured carrots by natural fermentation at ambient temperature. Microbiological and chemical characteristics of this product were studied for 15 days from the start of fermentation. The fermentation was found to be a typical lactic acid fermentation by *Leuconostoc mesenteroides*. The initial microbial counts in the brine varied from 10^3 to 10^5 cfu ml^{-1}, which increased to a maximum value of 10^7 cfu ml^{-1} as the fermentation progressed. Initial microbial load was due to soil bacteria belonging to *Bacillus* spp. and *Pseudomonas* spp. After four days of start of fermentation, the fermentation was complete, acidity and pH were 0.3% and 4.0 respectively.

Keywords : Carrots, Fermented beverage, *Kanji.*

Preservation of vegetables by fermentation, not only improves nutritive value and palatability, extends seasonal availability and prolongs shelf life but also renders them microbiologically safe. Many studies have been conducted to determine the physical, chemical and microbiological changes during fermentation of cabbage and cucumber (Ethchell et al. 1964; Pederson and Albury 1969; Pederson 1978) and most of these fermentations have been carried out by heterofermentative lactic acid bacteria like *Leuconostoc mesenteroides, L. plantarum* and *L. brevis* (Pederson 1978). Orillo et al (1969) studied the lactic acid fermentation of various blends of vegetables some of which contained a proportion of carrots also. Ramdas and Kulkarni (1987) preserved sliced carrots in 2% brine to get an acceptable product. A special fermented beverage traditionally known as *Kanji* was prepared from crimson coloured carrots supplemented with salt and mustard powder. Although attempts have been made to standardise the method of fermentation of *Kanji* (Sethi 1990; Ramdas and Kulkarni 1987), no systematic study has been reported with respect to chemical and microbiological changes that occur during fermentation. Therefore, the present investigations were conducted to determine the microorganisms involved and the chemical changes brought about by these microorganisms during carrot fermentation.

Preparation of Kanji *: Kanji* was prepared by the modified method of Ramdas and Kulkarni (1981). Carrots (crimson coloured) were procured from the local market and washed thoroughly in running tap water to remove adhering particles. These were sliced into pieces of 3-4 cm length and 1-2 cm thickness after peeling and rinsing with sterile water. Fermentation of sliced carrots was carried out in 10 litre glass bottles containing 1.5 kg sliced carrots, 3 litres distilled water, 3% NaCl and 4% mustard powder at room temperature (20 ± 2°C) for 15 days. Samples of fermenting brine were drawn for bacteriological and chemical analysis daily for the first seven days and on the 10th and 15th days in the following week.

Standard plate counts : The brine samples were serially diluted and appropriate dilutions were plated on Man Rogosa Sharpe (MRS), nutrient agar and *acetobacter* media to determine the number of different types of microorganisms in the fermenting brine drawn at various intervals. The plates were incubated at 30°C, till microbial colonies appeared.

Morphological, biochemical and culture characterisation : Types of microbial colonies, cell shape and arrangement, Gram stain and spore stain were used to characterise the genera. Several bacterial isolates selected on the basis of morphological characters were also characterised for their ability to ferment different sugars, presence of catalase, production of dextran in presence of 5% sucrose and growth at different salt concentrations and temperatures at 5.6 pH.

Total and reducing sugars : Total and reducing sugars were determined colorimeterically by the methods described by Dubois et al (1956) and Honda et al (1982), respectively.

Acidity : Total acidity was determined by titrating an aliquot of brine sample against 0.02N NaOH to pH 8.3 using a pH meter (Niketic Aleksic et al. 1993), whereas volatile acidity was calculated as per cent acetic acid by titrating distillation product of an aliquot of brine sample against 0.02N NaOH using phenolphthalein as indicator (Kanwar and Chopra 1991).

Ethanol : The amount of alcohol produced during fermentation was determined by the method described by Caputi et al (1968).

On MRS medium, the number of bacteria was found to be 3.1×10^5 cfu ml^{-1} of brine at the start of fermentation, which increased to 1.5×10^7 cfu ml^{-1} after 5 days (Table 1). The

TABLE 1. ESTIMATED VIABLE BACTERIAL COUNTS IN FERMENTING BRINE SAMPLES DURING DIFFERENT STAGES OF FERMENTATION

Days of fermentation	Man Rogosa Sharp medium	Nutrient agar medium	*Acetobacter* medium
0	3.1×10^5	2.8×10^3	–
1	1.6×10^6	1.0×10^4	–
2	2.2×10^6	1.0×10^4	–
3	2.4×10^6	1.1×10^4	–
4	8.2×10^6	1.0×10^4	1.0×10^5
5	1.5×10^7	1.2×10^4	1.3×10^5
6	1.6×10^7	1.3×10^4	1.5×10^5
7	3.2×10^7	1.4×10^5	1.6×10^5
10	3.4×10^7	1.7×10^5	1.8×10^5
15	2.5×10^6	2.1×10^5	1.2×10^5

* *Corresponding Author*

TABLE 2. MORPHOLOGICAL, CULTURE AND PHYSIOLOGICAL CHARACTERISTICS OF BACTERIA ISOLATED FROM FERMENTED CRIMSON COLOURED CARROTS ON MRS MEDIUM

	Isolate 1	Isolate 2	Isolate 3
Colony characteristics	Circular, raised, white and small	Circular, raised, colourless and small	Circular, raised colourless and small
Cell morphology	*Coccobacilli* (pairs or small chains)	*Cocci* (pairs or tetards)	*Coccobacilli* (pairs or small chains)
Gram reaction	+	+	+
Spore formation	–	–	–
Catalase test	–	–	–
Sugars fermentation			
Arabinose	–	–	–
Ribose	±	+	±
Xylose	±	+	+
Glucose	+	+	+
Fructose	+	±	+
Sucrose	+	+	+
Maltose	+	+	±
Cellobiose	–	–	–
Lactose	+	+	±
Effect of salt concentration			
4.0%	+	+	+
6.5%	+	+	+
8.0%	–	±	–
Growth temperature			
15°C	+	+	+
37°C	+	+	+
45°C	–	±	–
Production of dextran in presence of 5% sucrose	–	–	+
Probable Genus	*Leuconostoc mesenteroids*	*Pediococcus* spp.	*Leuconostoc dextranicum*

bacterial count remained constant thereafter. Three types of colonies were distinguished on the basis of colony characteristics. These were later on confirmed to be distinct on the basis of morphological and biochemical studies conducted with pure culture isolates (Table 2). Isolate numbers 1 and 3 were present throughout the fermentation. In contrast to microbial succession of Sauerkraut fermentation during which fermentation initiated by *L. mesenteroides* is followed by *Lactobacillus brevis*, *Pediococcus cerevisiae* and *L. plantarum*, *Leuconostoc mesenteroides* and *L. dextranicum*, tolerant to low salt concentration (6.5%), were predominant bacterial species during natural fermentation of carrots. Isolate number 2 was observed occasionally So. *Pediococcus* probably did not play any important role in carrot fermentation. According to Pederson and Albury (1953) also, the best results were usually obtained from fermentations carried out by *Leuconostoc mesenteroides*.

One type of yeast, which did not survive for long, was also observed at the start of fermentation. This might be due to selective anti-microbial activity of mustard powder against yeast which was added during *Kanji* preparation (Sethi and Anand 1984).

On nutrient agar, two types of bacterial colonies were observed in fermenting brine at the start of fermentation. Morphologically, these were found to be non-spore forming, gram-negative, small rods of *Pseudomonas* spp. and spore forming, gram-positive, large rods of *Bacillus* spp. However, these colonies disappeared after the 4th day, because of high lactic acid content in the brine produced by the predominant lactic acid bacterial species. Such types of surface microorganisms belonging to the genus *Pseudomonas*, *Aerobacter*, *Achromobacter* and *Flavobacterium* have also been reported on the surface of the leaves of cabbage (Pederson 1978). On the 4th day, small, white, pinheaded, raised colonies were observed and counted to be 1 x 10^4 cfu ml^{-1} of brine sample (Table 1). These colonies increased to 1.4 x 10^5 cfu ml^{-1} after 7 days. Such types of colonies were also observed on *acetobacter* medium on the 4th day and afterwards. The number of these bacterial colonies remained constant (~10^5 cfu ml^{-1}) from 4-15 days. Morphologically, these were found to be gram-negative small rods typical of acetic acid bacteria.

Biochemical changes during fermentation : Total sugars in fermenting brine were found to be 114 µg ml^{-1} after 24 h of the start of fermentation. This increased to 200 µg ml^{-1} after three days and decreased thereafter (Fig. 1). The decrease in total sugars was steep from 200 µg ml^{-1} to 30 µg ml^{-1}, indicating a fast rate of fermentation between 3rd and 4th days of fermentation. This level of total sugars was maintained throughout the fermentation. The amount of reducing sugars also increased to a maximum level of 90 µg ml^{-1} on the 3rd day and then decreased.

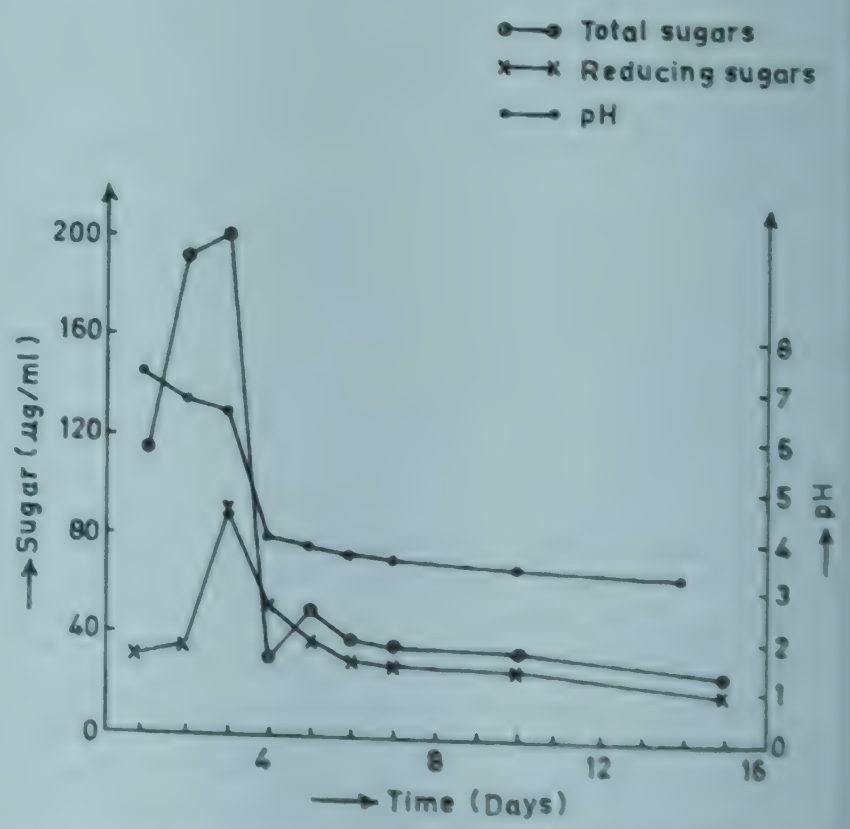

Fig. 1 Total sugars, reducing sugars and pH of fermenting brine at different time intervals

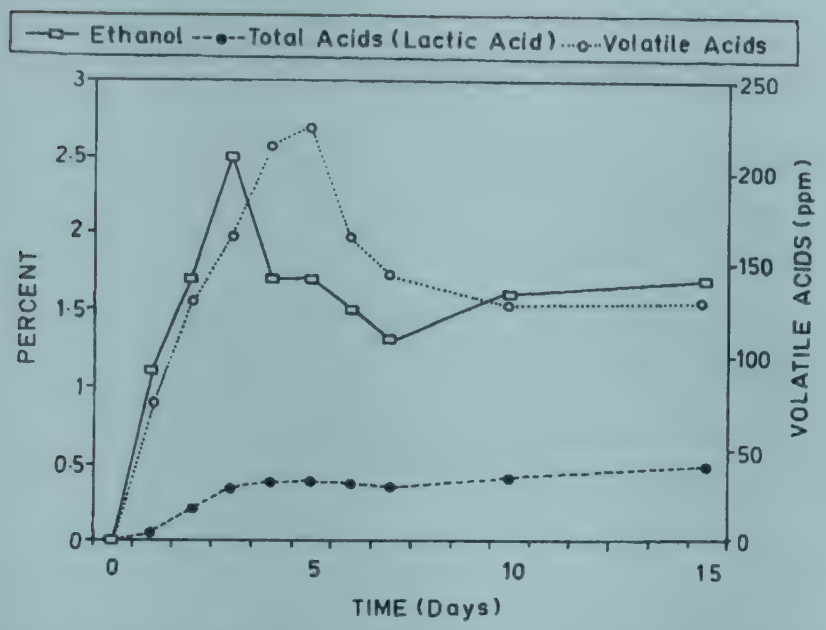

Fig. 2. Total acidity (expressed as % lactic acid) ethanol % and volatile acids (ppm) in fermenting brine at different time intervals.

As the reducing sugars were utilized, the organic acids were produced and the pH of the brine dropped from 7.2 to 4.0 on the 4th day. The total acidity on the 4th day was found to be 0.39% (Fig. 2). With the increase in population of lactic acid bacteria, there was an increase in the rate of lactic acid production between 3rd and 4th days that led to high acidity and steep fall in pH on the 4th day. The total acidity increased to 0.48% and pH decreased slowly to 3.3 after 14 days. In fermentation of whole carrots, an acidity of even 1.1-1.3% as lactic acid has been reported by Niketic-Aleksic et al (1973). Similarly, the amount of lactic acid has been found to be 1.8-2.2% (Pederson et al. 1962) in completely fermented Sauerkrat. Thus, beverage *Kanji* was less acidic as compared to Sauerkrat and fermented whole carrots. Like Sauerkrat fermentation, production of ethanol was also observed during carrot fermentation (Fig. 2). The concentration of ethanol increased slowly to 2.5% after three days and then decreased due to oxidation to acetic acid by acetic acid bacteria, which were present in sufficiently high number in brine on the 4th and 5th days (Table 1) of fermentation, leading to a maximum level of volatile acidity of 225 ppm (Fig. 2).

The fermentation of carrot slices was complete and fermented beverage called *Kanji,* without any scum formation, was ready for consumption within four days having an acidity of 0.3% and pH of 4.0. Thus, *Kanji* fermentation is a typical lactic acid fermentation.

Kanji, the fermented beverage has the potential for commercial exploitation. Therefore, further investigation with regard to preservation and improvement in fermentative characteristics are required to be conducted.

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Rheology of Cooked Decorticated Pulses

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A study was conducted on the rheology of three cooked decorticated pulses (*Mungi*, *Masar* and *Mah*) at different temperatures (20 to 50°C) and concentrations (15 to 25%) using a rotational viscometer. The results showed that cooked *Mungi dhal* had the maximum consistency coefficient, followed by *Mah* and *Masar*. The consistency coefficient increased with increase in concentration but decreased with increase in temperature. The regression model computed could be used to predict the consistency co-efficient of the cooked *dhal*, when the concentration and temperature are known. The effect of concentration of the consistency co-efficient was more significant than the effect of temperature in all the three *dhals*. The activation energy of flow for the various cooked *dhal* samples ranged between 20.52 and 54.01 kJ/g mol.

Keywords : Consistency index, Activation energy, *Dhal*.

Dhal is widely consumed by people in India. The term *dhal* is used for split grams and lentils. *Dhals* are good and cheap sources of proteins (Achaya 1994). These decorticated pulses are cooked into thick or thin gruels of various concentrations, depending on the liking of people in different parts of the country. *Dhals* are also combined in diverse ways with cereals to prepare *khichdi, idli, vada, dhokla*, etc. Design of equipment for fluid flow and heat transfer operations involved in the manufacture of canned cooked *dhal* require data on the rheological properties of this liquid food. Although the flow properties of a large number of liquid foods are known (Rao 1977), information on the rheology of these cooked decorticated pulses is not available. Therefore, the present investigation was carried out to determine the effect of temperature and concentration on the rheology of cooked *dhals*.

Three decorticated pulses viz. *Mungi (Phaseolus mungo), Masar (Lens culinaris)* and *Mah (Phaseolus vulgaris)* were purchased from the local market. Preliminary trials were carried out to determine the optimum cooking time for each *dhal*. *Dhal* (100 g) and water (300 ml) were mixed and cooked in a 4 litre Hawkins Pressure cooker (15 psi) for 6.5, 5.5 and 9 min for *Mungi*, *Masar* and *Mah*, respectively. The cooked *dhal* was diluted to 15, 17.5, 20, 22.5 and 25% concentrations by adding distilled water. Viscosity and shear stress were measured at different shear rates using Brookfield Viscometer (Brookfield Engineering Inc. model DV II). A 500 ml beaker with a diameter of 8.5 cm was filled upto a height of 8.5 cm with the cooked *dhal* sample and brought to the desired temperature in TC-500 water bath (Brookfield Engineering Inc.). Measurements were taken 2 min after the spindle (no. 18) was immersed in the sample so as to allow thermal equilibrium in the sample and to eliminate the effect of immediate time dependence. Log-log plots of shear stress vs shear rate were plotted to determine the consistency index (k) and flow behaviour index (n) using power law equation (Rao 1977; Ibraz and Pagan 1987).

$$\tau = k(dv/dy)^n$$

where τ = shear stress (Nm^{-2}), k = consistency index (Nsm^{-2}) and dv/dy = shear rate (s^{-1}).

Fig 1 to 3 show that the viscosity of the cooked *dhal* decreased with increasing shear rate and temperature. *Mungi dhal* showed the maximum viscosity, followed by *Mah* and *Masar* (Fig 4). The flow behaviour indices (n) of all cooked *dhal* samples at different concentrations and temperatures varied

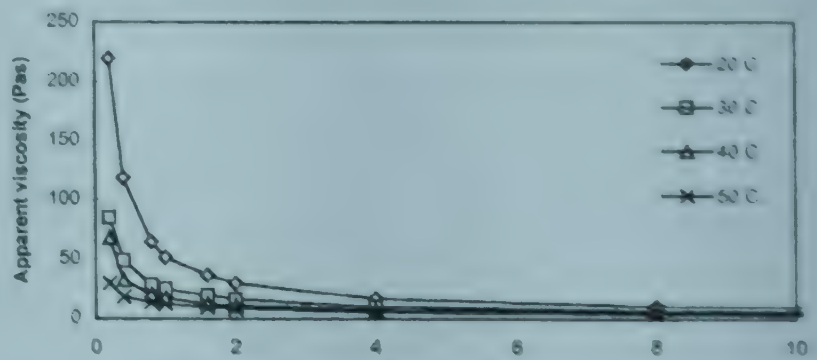

Fig. 1. Effect of temperature on the apparent viscosity of cooked *Mungi dhal* (25%)

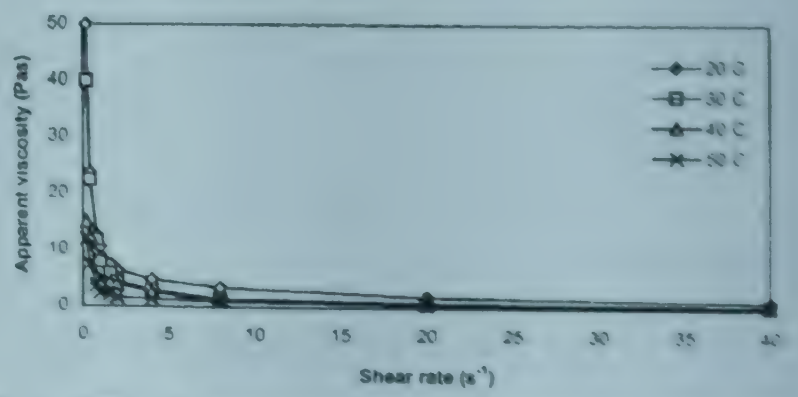

Fig. 2. Effect of temperature on apparent viscosity of cooked *Masar dhal* (25%)

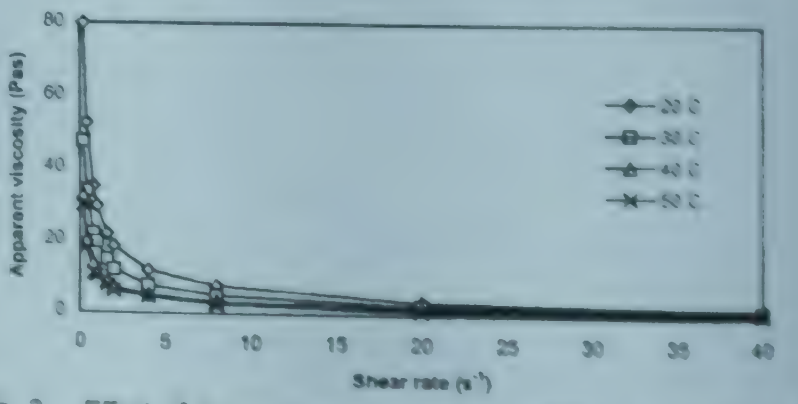

Fig. 3. Effect of temperature on apparent viscosity of cooked *Mah dhal* (25%)

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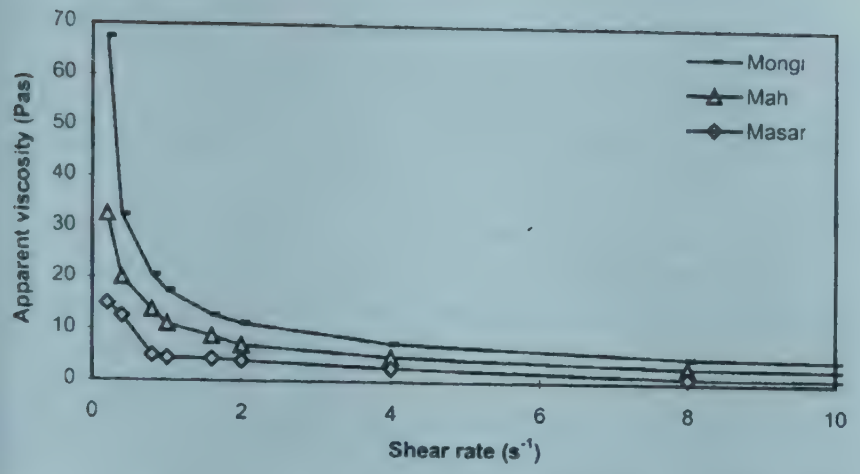

Fig. 4. Effect of shear rate on apparent viscosity of cooked *dhal* (25%) at 40°C

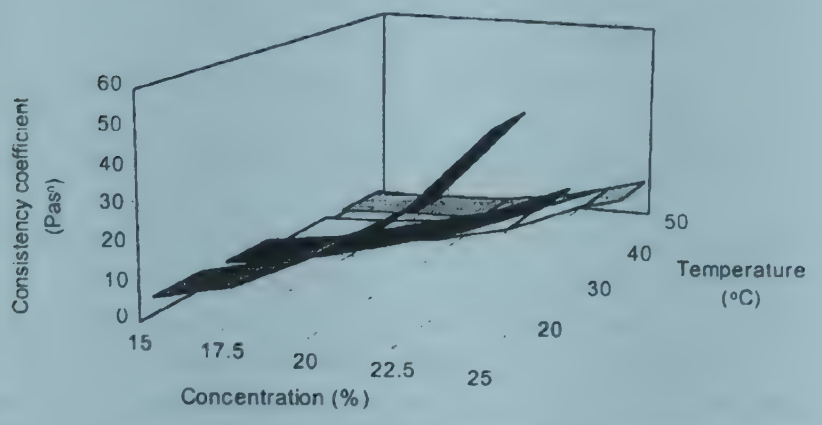

Fig. 5. Consistency coefficient as a function of concentration and temperature of cooked *Mungi dhal*

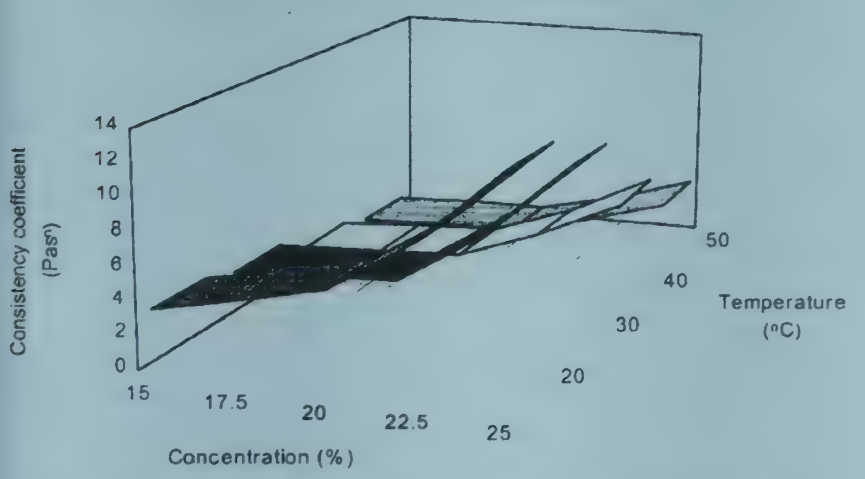

Fig. 6. Consistency coefficient as a function of concentration and temperature of cooked *Masar dhal*

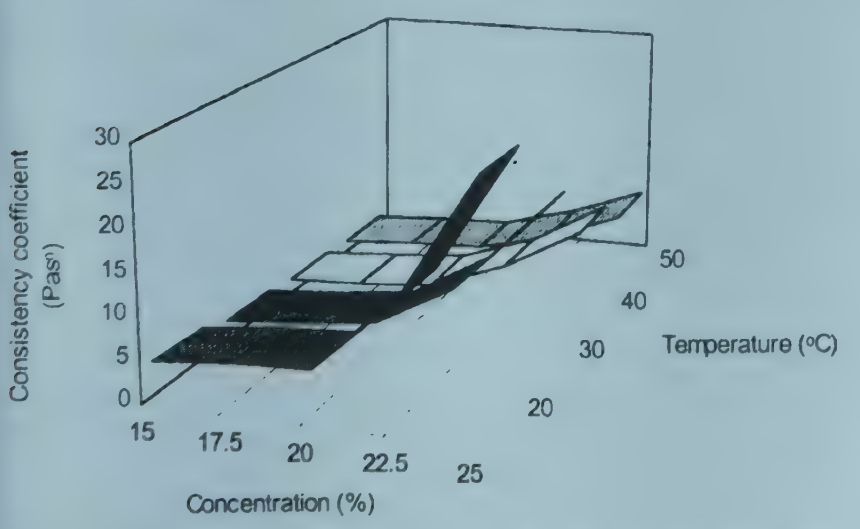

Fig. 7. Consistency coefficient as function of concentration and temperature of cooked *Mah dhal*

from 0.13 to 0.46, indicating their pseudoplastic nature. The effect of temperature and concentration can be combined into a single logarithmic model for simulation purposes.

$$\ln k = \alpha + \beta T^{-1} + \gamma C$$

Employing the natural log of consistency index, the *dhal* concentration and temperature, the magnitudes of co-efficients of the above equation were estimated by multiple linear regression.

$$\ln k = -4.068 + 52.923\ T^{-1} + 0.2216\ C_1 \quad \text{..... (1)}$$

$$\ln k = -3.1602 + 42.274\ T^{-1} + 0.1478\ C_2 \quad \text{..... (2)}$$

$$\ln k = -3.2198 + 30.174\ T^{-1} + 0.192\ C_3 \quad \text{..... (3)}$$

where k = consistency index (Nms^{-2}), T = temperature in °C and C_1 and C_2 and C_3 = the concentrations of *Mungi, Masar* and *Mah*, respectively. The R^2 values were 0.956, 0.896 and 0.940 for equations 1, 2 and 3, respectively. The R^2 values indicate that the regression equations can be used to predict the consistency index, when the temperature and concentration are known. Similar equations have been derived for consistency index and apparent viscosity for orange juice (Vitali and Rao 1984) and for reconstituted milk (Reddy and Datta 1994). Concentration causes an increase in the consistency index, whereas temperature has a reverse effect as shown in three-dimensional Fig 5 to 7. The consistency indices of *Mungi* decreased by 74.8, 79.6, 86.3, 81.1 and 78.39% at 15, 17.5, 20, 22.5 and 25% concentrations when the temperature increased from 20 to 50°C, whereas in *Masar,* percent decreases in consistency indices were 71.05, 74.8, 79.2, 74.3 and 71.5% and in *Mah,* the values were 55.6, 55.4, 50.19, 63.37 and 70.06% at 15, 17.5, 20, 22.5 and 25%, respectively with increase in temperature from 20 to 50°C. The consistency indices of 55.08, 13.16 and 28.96 (Nsm^{-2}) were highest at 25% concentration and at 20°C for *Mungi, Masar* and *Mah* and the values of 1.29, 0.88 and 1.6 (Nsm^{-2}) were least at 15% concentration and at 50°C. The F-values in Table 1 indicate that the effect of concentration on the consistency co-efficient was

TABLE 1. ANALYSIS OF VARIANCE OF EFFECT OF TEMPERATURE AND CONCENTRATION OF CONSISTENCY CO-EFFICIENT

	Sum of squares	Degrees of freedom	F-calculated	P-value	F-value
Mungi					
Concentration	1627.26	4	8.403	0.0018	3.259
Temperature	1136.02	3	7.822	0.0037	3.490
	580.89	12			
	3344.14	19			
Mah					
Concentration	593.46	4	12.289	0.0003	3.259
Temperature	158.48	3	4.375	0.0267	3.490
	144.87	12			
	896.82	19			
Masar					
Concentration	117.97	4	15.816	0.00009	3.259
Temperature	74.58	3	13.330	0.00039	3.490
	22.37	12			
	214.93	19			

TABLE 2. ACTIVATION ENERGY OF COOKED *DHALS* AT DIFFERENT CONCENTRATIONS

Dhal	Conc. %	Activation energy, Ea (kJ/g mol)	Arrhenius constant, ko (Pas^n)	R^2
Mungi	15.0	38.990	6×10^{-7}	0.949
	17.5	44.161	1×10^{-7}	0.969
	20.0	54.018	5×10^{-8}	0.984
	22.5	48.224	4×10^{-7}	0.999
	25.0	39.114	5×10^{-6}	0.984
Masar	15.0	33.500	4×10^{-6}	0.961
	17.5	35.881	2×10^{-6}	0.990
	20.0	40.411	3×10^{-7}	0.995
	22.5	38.989	4×10^{-6}	0.981
	25.0	33.870	1×10^{-5}	0.955
Mah	15.0	24.275	0.0002	0.918
	17.5	21.571	0.0007	0.969
	20.0	20.527	0.0012	0.993
	22.5	27.152	0.0002	0.995
	25.0	32.704	4×10^{-5}	0.996

more significant than the effect of temperature in all the three *dhals*. The effect of concentration was more prominent in *Mah* followed by *Masar* and *Mungi*.

The effect of temperature on the consistency of cooked *dhal* was described by the Arrehenius relationship (Savaracos 1970; Rao et al. 1984) as follows :

$$k = k_o \exp(E_a/RT)$$

where k_o = the Arrhenius constant (Pas^n), E_a = the activation energy (kJ/gmol) of flow, R = the gas constant and T = the absolute temp in K. The activation energy values of flow for the three cooked *dhals* are given in Table 2. The activation energy values were maximum for *Mungi*, followed by *Masar* and *Mah*.

Consistency index was found to be directly proportional to concentration and inversely proportional to temperature. It was concluded from the present study that cooked *Mungi dhal* had the maximum consistency index and activation energy of flow, when compared to *Masar* and *Mah*. The data reported could be of use to the food industry engaged in the canning of these cooked decorticated pulses.

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RESEARCH NOTE

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Effect of Extraction Parameters on the Properties of Fenugreek Mucilage and its Use in Ice Cream as Stabilizer

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The yield of mucilage obtained after soaking fenugreek flour in water (1:10 w/v) for 20 min. at 30°C, 50°C or 75°C, followed by extraction with water (1:50 w/v) for 2 h at pH 2, 5, 7, 9 or 11 with continuous stirring was found to be 30-32%. Mucilage extracted at pHs 2 and 11 had significantly higher carbohydrate contents, thereby indicating comparatively more purified mucilage. Mucilage extracted at pH 11 from fenugreek flour soaked at 50°C had significantly higher values for water holding capacity, fat binding capacity, emulsion activity, emulsion stability, foaming capacity, foam stability and solubility. The results revealed that the best quality ice cream with optimum mix viscosity, over run and melt down time alongwith higher sensory scores could be prepared by the use of 0.2% mucilage extracted at pH 11 or 2 as stabilizer.

Keywords : Fenugreek flour, Fenugreek mucilage, Functional properties, Ice cream stabilizer.

Fenugreek *(Trigonella foenum - graecum* L.) is an annual herb, which is being cultivated in the Mediterranean area in India and North Africa. Fenugreek seeds are known to contain about 20% mucilagenous matter (Rao and Sharma 1987). The mucilage is a galactomannase highly branched with basic linkages similar to guar, carbs and kentucky coffee bean gums (Smith and Montogomery 1959). The functional properties of fenugreek flour and powder have been studied. The water and fat absorption and oil emulsification of powder were found to be higher but nitrogen solubility was lower than the parent flour, suggesting denaturation of proteins during preparation of powder (Abdel - Aal et al. 1985, 1986). Various seeds gums/mucilages have been tried as thickeners, emulsifiers, stabilizers and foaming agents in various food applications. Baig et al (1992) used mustard seed mucilage as stabilizer in ice cream, which could mimic the property of gelatin. The present investigation was undertaken to study the optimization of process parameters for extraction of mucilage of desirable functional quality and its use in ice cream as stabilizer.

Fenugreek seeds (variety - 'Pusa', early bunches) were procured from Horticultural Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Preparation of flour : The seeds were cleaned, washed and dried to moisture level of less than 10%. The dried seeds were ground in a Pin mill (Ganson's India Ltd. Mumbai) and the whole ground flour was passed through an 80 mesh sieve.

Extraction of mucilage : The flour was suspended in water in the ratio of 1:10 (w/v) and incubated in a water bath for a period of 20 min at a temperature of 30°C, 50°C or 75°C in order to complete hydration and swelling of the flour. The flour : water ratio was then adjusted to 1:50 (w/v) by adding additional amount of distilled water. The slurry was divided into five lots and pH values of these lots were adjusted to 2, 5, 7, 9 or 11. The contents were stirred on an electrically operated magnetic stirrer for 2 h at 30°C. The suspension was filtered through a double layered muslin cloth and the filtrate was then mixed with equal volume of 95% ethanol to precipitate mucilage. After 5 min. the precipitate was collected over a muslin cloth and dried under vacuum at 30°C for 1 h to remove residual alcohol. Finally, the mucilage was freeze-dried to a moisture level of 7-8%.

Preparation of ice cream : Ice cream samples were prepared using the method of Baig et al (1992). The ingredients were mixed so as to give 13% fat. 10% milk solids-not-fat. 15% sugar and 0.1%, 0.15%, 0.2% or 0.25% fenugreek mucilage as stabilizer in the finished product. The ice cream of same composition with 0.4% gelatin as stabilizer was taken as control for comparison.

Analysis : Proximate composition of fenugreek mucilage was determined by AOAC (1984) methods. Water holding capacity and fat binding capacity of mucilage samples were determined by methods described by Quinn and Paton (1979) and Lin et al (1974), respectively. Emulsion and whipping properties of mucilage were estimated using the method of Yasumatsu et al (1972). Solubility of mucilage was determined by the method of Ma (1983). Viscosity of ice cream mix was determined by Synchro electric viscometer model LVT (Brookfield USA). Over run % of ice cream was calculated by dividing the difference between weights of 1 litre mix and 1 litre ice cream by weight of 1 litre ice cream multiplied by 100. Melt down time of ice cream was recorded by noting the time (min) required for complete melting of 100 g ice cream at 30°C, placed on a glass slide resting on a funnel. Sensory qualities of ice cream were evaluated by 10 panelists on a 9-point Hedonic scale. The results were analysed statistically using the technique of analysis of variance (Snedecor and Cochran 1967).

Trials were conducted to optimize the stirring time for the extraction of crude mucilage from fenugreek flour. It was found that an extraction time of 2 h was optimum for obtaining maximum yield (30 to 32%) of crude mucilage. Shankaracharya and Natarajan (1972) have reported that the fenugreek meal contains about 50% of indigestible hydrophilic mucilage. El-Madfa and Kunl (1976) and International organization for standardization (1982) have reported that fenugreek seeds contain 28% mucilage. Rao and Sharma (1987) found 20% mucilage in fenugreek seeds. The differences in these reports may be attributed to the method of extraction employed and extent of purity of mucilage obtained.

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TABLE 1. EFFECT OF SOAKING TEMPERATURE AND EXTRACTION pH ON THE PROXIMATE COMPOSITION OF FENUGREEK MUCILAGE

Temperature of soaking of flour in water, °C	pH of extraction of mucilage	Proximate composition of mucilage			
		Fat, %	Proteins, %	Ash, %	Carbohydrates, %
30	2	3.91	12.24	4.93	78.92
	5	4.35	15.50	3.78	76.37
	7	4.60	15.21	3.65	76.54
	9	4.25	15.42	3.75	76.58
	11	3.12	12.35	5.47	79.06
50	2	3.65	11.45	5.16	79.73
	5	4.05	14.57	4.35	77.03
	7	4.23	15.10	4.46	76.21
	9	2.92	15.03	3.87	78.18
	11	2.45	11.28	5.70	80.57
75	2	3.50	11.67	5.45	79.38
	5	4.03	13.50	4.45	78.02
	7	3.90	14.22	5.10	76.78
	9	3.46	14.25	5.35	76.84
	11	2.26	10.96	5.89	80.89
CD at 1% level of significance					
Soaking temperature		0.384	0.353	0.405	0.377
Extraction pH		0.496	0.456	0.523	0.487

Extraction time : 2 h

The mucilage samples extracted at pHs 2 and 11 had significantly ($P<0.01$) lower fat and protein and higher ash and carbohydrate contents as compared to those extracted at pHs 5, 7 and 9 (Table 1). On increasing the temperature of soaking of fenugreek flour in water prior to the extraction of mucilage from either 30°C to 50°C or 50°C to 75°C, the protein content decreased significantly ($P<0.01$), whereas ash and carbohydrate contents increased. However, no significant change was observed in fat % of mucilage. The high temperature of soaking of fenugreek flour and extremes of pH used for extraction of mucilage probably resulted in partial denaturation of proteins, thereby decreasing the protein content of mucilage. The values of proximate composition of fenugreek mucilage obtained in the present investigation were similar to those reported by Weber et al (1974) for mustard mucilage.

The values of functional properties increased significantly ($P<0.01$) by increasing the temperature of soaking from 30°C to 50°C. However, further increase in soaking temperature from 50°C to 75°C did not alter the values of functional properties significantly (Table 2). Therefore, 50°C was considered as an optimum temperature for the extraction of mucilage from fenugreek flour. The pH of extraction was also found to affect the functional properties of mucilage significantly ($P<0.01$). The maximum water holding capacity and emulsion capacity were found in mucilage extracted at pH 11, followed by mucilage extracted at pHs 7, 9, 2 and 5 (Table 2). The highest mean values for emulsion stability, foaming capacity and foam stability were also noted for mucilage samples extracted at pH 11, followed by those extracted at pHs 7, 9, 5 and 2. Abdel - Aal

TABLE 2. EFFECT OF SOAKING TEMPERATURE AND EXTRACTION pH ON THE FUNCTIONAL PROPERTIES OF FENUGREEK MUCILAGE

Temperature of soaking of flour in water, °C	pH of extraction of mucilage	Water holding capacity, ml water/g	Fat binding capacity, %	Emulsion activity, %	Emulsion stability, %	Foaming capacity, %	Foam stability, %	Solubility, %
30	2	13.5	62.0	70.2	60.4	15.0	7.4	65.4
	5	13.6	98.0	56.5	65.0	16.0	7.3	82.4
	7	18.0	75.5	65.0	75.5	25.0	15.1	68.0
	9	15.5	94.0	64.5	70.5	18.0	10.3	85.1
	11	24.5	92.0	99.0	98.0	28.0	20.3	91.4
50	2	17.5	97.6	91.3	65.0	16.1	9.2	69.5
	5	15.5	109.5	65.0	70.5	18.0	9.1	85.4
	7	20.5	81.1	69.5	80.0	30.0	21.0	71.4
	9	18.0	95.0	75.0	78.0	20.1	13.1	89.1
	11	25.0	95.0	99.6	99.0	30.0	25.1	94.9
75	2	17.6	98.0	91.5	65.4	16.1	9.4	70.0
	5	15.5	110.5	65.5	71.0	18.0	10.1	86.0
	7	20.5	80.5	71.0	80.0	30.0	21.5	72.0
	9	18.0	95.0	75.0	78.5	20.1	13.5	89.5
	11	25.5	95.0	99.5	99.0	30.2	25.9	95.0
CD at 1% level of significance								
Soaking temperature		0.408	0.699	0.724	0.736	0.772	0.502	0.541
Extraction pH		0.527	0.903	0.934	0.950	0.996	0.648	0.698

Extraction time : 2 h

TABLE 3. EFFECT OF EXTRACTION pH OF MUCILAGE AND ITS CONCENTRATION IN MIX ON THE QUALITY OF ICE CREAM

Extraction ph of mucilage	Concentration of mucilage in mix	Viscosity of mix, centipoise	Over run, %	Meltdown time, min	Sensory score
2	0.10	195.5	79.3	49.1	7.3
	0.15	210.6	85.1	51.2	7.8
	0.20	235.2	94.2	56.2	8.5
	0.25	275.4	95.2	60.1	7.5
5	0.10	180.2	70.1	45.1	7.0
	0.15	192.3	76.3	48.5	7.4
	0.20	211.8	79.2	50.4	8.1
	0.25	233.3	80.1	50.2	7.2
7	0.10	185.2	73.2	48.2	6.9
	0.15	199.2	77.1	49.3	7.4
	0.20	213.1	80.4	51.3	7.8
	0.25	243.2	82.1	53.4	7.2
9	0.10	182.2	72.2	45.5	7.0
	0.15	194.3	78.3	48.2	7.2
	0.20	209.4	82.2	50.4	7.5
	0.25	234.9	83.3	52.3	7.4
11	0.10	206.3	95.3	50.3	7.4
	0.15	234.1	105.2	55.3	8.0
	0.20	259.2	108.1	59.5	8.5
	0.25	325.3	110.2	63.2	7.4
CD at 1% level of significance					
Extraction pH		0.405	4.552	0.253	0.344
Concentration of mucilage in mix		0.362	4.071	0.226	0.307

Soaking temperature of flour : 50°C

Extraction time : 2 h.

et al (1986) found that water holding capacity, fat absorption, and oil emulsion capacity of fenugreek flour were 487%, 123% and 27%, respectively. The corresponding values reported for fenugreek powder were 352%, 190% and 37%.

The fat binding capacity of mucilage extracted at pH 5 was found to be highest, followed by mucilage extracted at pHs 9 and 11, 7, and 2 (Table 2). The solubility of mucilage in water was maximum, when it was extracted at pH 11, followed by those extracted at pHs 9, 5, 7 and 2. Mazza and Biliaderia (1989) studied the solubility of 0.5% solution of flax seed mucilage and found it to be 70-90%. They further reported that the solubility increased with increase in temperature of suspension. In the present investigation, the solubility values ranged from 65 to 95%, depending upon the temperature and pH of extraction.

On increasing the concentration of mucilage in ice cream mix from 0.1 to 0.25%, the viscosity and meltdown time of ice-cream increased significantly ($P<0.01$). However, the over run and sensory scores of ice cream increased significantly ($P<0.01$) only up to 0.2% concentration of mucilage in the ice cream mix (Table 3). Further increase in mucilage concentration to 0.25% caused a significant ($P<0.01$) decrease in sensory scores, but the effect on over run was non-significant. Baig et al (1992) also observed that the increasing concentration of mustard mucilage as stabilizer in ice cream increased mix viscosity and melting resistances. They also found that 0.2% level of mucilage gave better quality ice cream than 0.5% gelatin.

A significant ($P<0.01$) high viscosity of ice cream mix and meltdown time of ice cream was obtained by using mucilage extracted at pH 11, followed by the mucilage extracted at pHs 2, 7, 9 and 5. The mucilage extracted at pH 11 also gave ice cream with significantly ($P<0.01$) high over run, followed by one prepared from mucilage extracted at pH 2 (Table 3). The mucilage extracted at pHs 5, 7 and 9 yielded ice cream with nearly same over run. Sensory evaluation of ice cream samples revealed that best quality ice cream could be obtained by using mucilages extracted at pH 11 or 2 as stabilizers. The other ice cream samples prepared by using mucilages extracted at pHs 5. 7 and 9 were observed to be significantly ($P<0.01$) inferior in sensory quality.

Thus, ice cream with higher sensory scores, over run, viscosity and meltdown time can be prepared by using fenugreek mucilage extracted at pH 11 or 2 at a level of 0.2% as a stabilizer.

The results of the present studies have shown that a good quality mucilage could be obtained by aqueous extraction of fenugreek flour at pH 11 for 2 h after soaking in water for 20 min at 50°C. Mucilage thus obtained had significantly superior functional properties and may be used satisfactorily in ice cream as a stabilizer of vegetable origin.

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RESEARCH NOTE

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Utilization Possibilities of Jellyfish *Rhizostoma pulmo*, As a Food in the Black Sea

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Jellyfish, *Rhizostoma pulmo*, from the Black Sea were processed according to Maruichi and Wootton processing methods and stored at – 4°C for two different periods, i.e., 1 and 24 months. Moisture, ash and protein values were determined. In addition, the levels of calcium, magnesium, copper, iron, zinc and nickel were determined in the salted jellyfish. Since the ash and protein contents were determined after removing the excess salt and alum from dried samples, the values appeared to be quite different from those previously reported. In conclusion, both the processing methods as well as the storage periods were found to be effective in giving acceptable products.

Keywords : *Rhizostoma pulmo*, Black sea, Salted jellyfish, Processing method, Storage period, Chemical composition.

There are several gelatinous Cnidaria such as *Rhizostoma pulmo, Aurelia aurita* and Ctrenophore *(Mnemiopsis leidyi* and *Pleurobrachia pileus)* species in the Black Sea. Although, there is not many published work on biomass of *R. pulmo,* total biomasses (wet weight) of *A. aurita* and *M. leidyi* have been reported to be 350-900 millions and 0.8 – 1.0 billion tonnes (Shuskina and Musayeva 1983; Mee 1991; Vinogradov and Tumantseva 1993). This high biomasses of gelatinous macrozooplankton is creating serious problem in fishing for anchovy, the main commercial species in the Black Sea, by clogging the meshes of purse-seine. During peak fishing season of anchovy (November-December), individual weight and total biomass of *R. pulmo* reach its maximum values (Netchaett and Neu 1940). In addition to time spent to clean the clogged purse-seines, there is a danger of net tearing due to overloading of target fish and jellyfish (Ozer 1994). These species have not got any natural predator and fishing pressure. As a result, their biomasses are increasing rapidly in eutrophic waters of the Black Sea. During recent years, a few authors (Mee 1991; Vinogradov and Tumantseva 1993) claimed that the effects of these carnivore species on zooplankton and ichthyoplankton communities could be far more greater than those of all anthropogenic factors. Therefore, there seems to be an urgent need to control the biomass increase in the ecosystem and exclusive jellyfish fishing might be the only alternative.

Jellyfish species has been utilized as processed (salted) food in some Far East countries, particularly Japan, Korea and China. They import processed jellyfish from countries such as Malaysia, Thailand and Indonesia. Although the annual salted jellyfish production of Japan is around 7000 tonnes, this is far from compensating the domestic demand and Japanese are importing quite a substantial amount of (around 13000 tonnes/ year) salted jellyfish (Soonthonvipat 1976; Morikawa 1984; FAO 1993). Previous studies on utilization of jellyfish have shown that members of Rhizostomeae order, including *R. pulmo,* (Mayer 1910; Omori 1981; Morikawa 1984) with firm structure and large size are suitable for commercial processing, but it has been reported that *A. aurita,* which is another abundant species in the Black Sea is not so favourable for commercial processing mainly due to its loose structure, small size and lack of crunchy texture in the final product (Sloan and Gunn 1985; Ozer 1994).

Although, several different methods have been used for processing the jellyfish (Soonthonvipat 1976; Omori 1981; Wootton et al. 1982; Sloan and Gunn 1985; Huang 1988), at present, only few of them are used in practice and the most common and best known one is Maruichi method, which consists of six phases and can be completed in 19 days (Krishnan 1984; Govindan 1984; Sloan and Gunn 1985). This method has been modified by Huang (1988) and two different methods were developed. In addition, Wootton et al (1982) have changed the salt (30-35%) and alum (1.5-3.0%) ratios used by Iljas and Arifuddin (1971) and developed a new method to be carried out in 5 phases and lasts 35 days. The salt and alum ratios of Wootton et al (1982) were 25-35% and 2-10%, respectively. Another worker (Suelo 1988) has tried to shorten the processing time of Wootton et al (1982) from 35 days to 10 days to develop a rapid processing method and concluded that this was not a suitable processing technique at least under Australian conditions. In these studies (Wootton et al. 1982 Huang 1988), various storage periods have been tested and biochemical composition of final products were determined. As far as the Black Sea area is concerned, there is no published study on the utilization of jellyfishes.

Therefore, the main aim of this study was to evaluate the possibilities of utilizing *R. pulmo,* which has got natural predator in the Black Sea and breaking the pelagic trophic link and creating serious problems in anchovy fishing. To determine the suitable processing method for *R. pulmo* and acceptable storage period for processed product, samples collected between August and December were processed according to two different methods, namely Maruichi and Wootton, and stored at –4°C for a month and 24 months.

Jellyfish : Individuals of *R. pulmo* with a diameter of over 30 cm were found around coast of Trabzon, south-eastern of

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Black Sea. Samples were collected by hand net and individuals larger than 30 cm were selected from those captured during anchovy fishing operations with purse-seine. Samples were transported to laboratory after they were placed in plastic containers with fresh sea water.

Preparation : Umbrellas and other body parts were separated by cutting off oral arms, gonads and intestines. After that, the umbrellas were uncurled, flattened and remains of manubrium portions were trimmed avoiding any damage to the surface of umbrella. Umbrellas were measured and weighed. These processes were completed in 6-8 h. The principle of the salting of jellyfish involves osmosis or simple diffusion (Krishnan 1984). Potassium alum and salt, normally used for fish brining, were used for processing.

Processing : As it has been mentioned previously, there are several different methods used in jellyfish processing and two of them viz., (i) Maruichi Shoji's processing method used commonly in Japan and (ii) the method developed by Wootton et al (1982) were followed during this study.

Processing with Maruichi Shoji method : According to this method, jellyfish was processed in 6 phases lasting for 19 days. The first five phases were of salting process for 15 days and the last phase was for salt-drying process for 4 days (Krishnan 1984; Govindan 1984; Sloan and Gunn 1985).

Chemical analyses : Moisture content was determined by drying processed samples at 60°C to a constant weight (5-7 days) in an oven (Omori 1969, 1978; Bamsted 1981). After removing the excess of salt and alum from pre-dried samples, which were crushed and weighed, the ash content was determined by keeping the samples in muffle furnace for about 24 h at 550°C to a constant weight and the results were expressed as % of dry weight. Protein content was determined using Kjeldahl method was used of AOAC (1980). The conversion factor N x 6.25, and the value was expressed as % of dry weight. After the samples were ashed, remains were dissolved 2 ml HCl and 25 ml distilled water (Mayzaud 1975) and Ca, Mg, Zn, Fe, Cu and Ni were analyzed using a Perkin-Elmer Atomic Absorption Spectrophotometer (model 403). For each mineral, wave lengths and light bulbs recommended by the manufacturing company (Perkin-Elmer 1971) were used and the results were expressed as mg/100g dry weight.

Statistical analysis : Analysis of variance and Tukey's test was used to determine statistical significance of differences between processing methods and storage periods (Minitab 1991).

In this study, jellyfish were processed using two main processing methods and stored at – 4°C for a period of either 1 or 24 months. A total of 78 jellyfish was processed and 25 of them were stored for a month and the rest for a period of 24 months. In addition, moisture, ash, protein, Ca, Mg, Cu, Fe, Zn and Ni contents of each sample, processed according to different methods and stored different periods were determined and compared. Results are presented according to processing methods and storage duration in Table 1.

As can be seen in Table 1, there were no significant differences between moisture content of products processed according to Maruichi's method and stored for two different periods. But Wootton method's storage period and both processing methods had significant effects on moisture contents (Table 1). In general, moisture contents of the final products processed with the Maruichi's method were higher than those of Wootton's method. The reason for this was that salt (20% of umbrella weight) and alum (6.4% of umbrella weight) levels used during salting according to Maruichi's method were less than those of the Wootton's method. So, the jellyfish processed following this method drained less water i.e., the final product inluded more moisture than that of the second method.

TABLE 1. MOISTURE CONTENTS (MEAN±SD) OF JELLYFISH PROCESSED ACCORDING TO TWO DIFFERENT METHODS AND STORED FOR DIFFERENT PERIODS

Method	Storage period			
	n	24 months	n	1 month
Maruichi	13	80.08 (0.62)^{a*}	7	79.56 (0.87)^{a**}
Wootton	40	79.04 (1.53)^{b***}	18	74.98 (2.24)^{b***}

$^{a, b}$ Means with the same letter are not significantly different within each storage period ($p \leq 0.05$)

$^{*, **}$ Means with the same stars are not significantly different within each processing method ($p \leq 0.05$)

TABLE 2. ASH CONTENTS (MEAN±SD) OF JELLYFISH PROCESSED ACCORDING TO TWO DIFFERENT METHODS AND STORED FOR DIFFERENT PERIODS

Method	Storage period			
	n	24 months	n	1 month
Maruichi	13	52.12 (12.22)^{a*}	7	38.10 (7.82)^{a**}
Wootton	40	42.30 (10.17)^{b***}	18	40.22 (5.17)^{a**}

$^{a, b}$ Means with the same letter are not significantly different within each storage period ($p \leq 0.05$)

$^{*, **}$ Means with the same stars are not significantly different within each processing method ($p \leq 0.05$)

The ash content of processed jellyfish was determined on dry weight basis and the results were compared according to processing methods and storage periods (Table 2). Although ash contents of processed jellyfish with Wootton's method did not show any significant differences according to storage periods, the differences between storage periods for Maruichi's method was significant. In addition, the ash content was significantly different for products processed differently and stored for different intervals.

After moisture and ash contents were obtained, protein values were determined. Therefore, data presented here combined for methods and storage periods. The mean protein contents (% of dry weight) were 53.22% (3.93; n=7) and 56.80% (5.34; n=35) for Maruichi's and Wootton's methods, respectively, while they were 54.59% (3.10; n=17) for 24 months and 56.94% (5.99; n=25) for one month storage. No significant difference in protein contents of final products was noted between any of the processing methods and storage periods.

Mineral content ranges of processed jellyfish are given in Table 3. Analysis was carried out using 78 samples and values showed considerable variations.

TABLE 3. VARIATION IN MINERAL CONTENTS OF PROCESSED JELLYFISH (ALL DATA COMBINED), mg/100 g DRY WEIGHT

Mineral content	Ca	Mg	Cu	Fe	Zn	Ni
Minimum	2.10	15.33	0.49	8.59	0.92	0.00
Maximum	31.27	111.50	17.37	42.48	22.27	2.12

TABLE 4. MOISTURE, PROTEIN AND ASH CONTENTS OF FRESH AND PROCESSED JELLYFISH REPORTED BY VARIOUS AUTHORS

		Percent		
Jellyfish samples	Moisture	Protein	Ash	References
Fresh	95.80	[c]1.30	2.50	Wootton et al (1982)
Experimental	66.20	6.70	27.40	"
Commercial	73.10	4.00	22.30	"
Dried	63.20	9.30	24.80	"
Fresh	96.10	1.07	2.35	Huang (1988)
Experimental	67.87	5.68	24.89	"
Malaysian product	66.98	5.87	25.76	"
Chinese product	67.36	6.82	23.90	"
Fresh	96-97	1-1.5	2-3	Subasinghe (1992)
Processed	65-75	4-6	17-25	"
Fresh	97.80	–	1.67	This study
Maruichi's method	79.56	*10.88	*7.79	"
Wooton's method	74.98	*14.21	*10.06	"

[c] : Calculated

[*] : Determined after salt and alum removal

There are few studies by various authors on the processing and chemical composition of jellyfish (Tsusaka and Suyama 1965; Wootton et al. 1982; Kimura et al. 1983; Sloan and Gunn 1985; Huang 1988; Suelo 1988). Among these, Wootton et al (1982) processed umbrella of Australian jellyfish (*Catostylus* spp.) and summarized chemical composition of fresh and processed materials, while Huang (1988) worked on manubrium and umbrella of *Stomolophus meleagris.* In addition, Subasinghe (1992) reviewed chemical composition of both fresh and processed jellyfish. The results of the present and the previous studies are summarized in Table 4 for broad comparisons.

As shown in Table 4, moisture values of fresh and processed *R. pulmo* determined in this study were higher than those reported in literature. The possible reasons were : a) In these studies, different jellyfish species were used and moisture contents of their umbrellas varied with species, b) Black sea water was less saline and consequently dry weight values were lower in comparison to species living in oceanic waters (Schneider 1988), c) There were differences in processing methods, d) In previous studies, moisture contents were determined before storage, whereas in present study, it was determined after at least 1 month storage and this caused loss in weight and e) after processing, the products were stored at different temperatures. In the present study, this temperature was quite lower (– 4°C) than those of previous ones and this might have caused the loss in texture (Subasinghe 1992).

The ash values in the fresh and processed jellyfish were found to be lower than the previous studies (Table 4). This was because ash contents were determined after removing salt and alum remnants around dried jellyfish. In this way, important portions of inorganic matter were removed from samples. When ash was determined without removing salt and alum, ash values were 18.2% for Maruichi's and 21.5% for Wootton's processing methods. Even these values were still lower than those reported in previous studies (Table 4).

In contrast to ash values, protein values obtained in this study were higher than those of previous ones (Table 4). The reason for this was removing excess of salt and alum before protein analysis. When excess inorganic matter included in samples, protein values declined to 2.5-3.5% of wet weight.

Two studies reported the mineral composition of jellyfish; one by Ryzhkov et al (1983) and another by Huang (1988). In the former study, Se, Zn, Co, Fe, Hg, Ag, Pb and Sb contents of fresh *A. aurita* and *R. pulmo* in Azov Sea were determined. In the second one, Ca, Mg, Cu, Fe and Zn values in the manubrium and umbrella of *S. meleagris* were analyzed. In the present study, Ni was determined in addition to minerals. Huang (1988) showed that all the mineral values of processed *R. pulmo* were higher than those of *S. meleagris*. This high mineral content of *R. pulmo* may have arisen from relatively high heavy metal concentration of the Black Sea and possibility of salt and alum used for processing.

Processed products were stored at – 4°C for 1 and 24 months. Results showed that both the storage periods would not affect appearance, but jellyfish stored for 24 months appeared to be quite thinned out. There were several studies on the storage period of processed jellyfish. Wootton et al (1982) reported that the products could be stored satisfactorily for 1 month at both 5°C and 25°C, whereas Huang (1988) stored the products at 10°C for 1 and 6 months satisfactorily, but the latter author found indications of textural breakdown in products stored at 25°C for 2 months.

Conclusion

Jellyfish *R. pulmo,* which is one of the abundant jellyfish species in south-eastern Black Sea coast, seems to be suitable for processing and storage. When two processing methods were compared, there were no significant differences in acceptability of the final product. But, Maruichi's processing method, which has shorter processing duration (19 days) and requires less salt and alum can be preferred to Wootton's method, lasting 35 days and requiring much more salt and alum. Although it is possible to store processed products at – 4°C for a month or 24 months without any deterioration in quality of product, storing one month appears to be more reliable because processed jellyfish are getting thinner when stored for 24 months.

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RESEARCH NOTE

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Limitations of Jaggery for Inversion as Compared to Sucrose

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Inversion of jaggery using acid and enzyme was carried out and compared with sucrose inversion. Hydroxy-methyl-furfural (HMF), the neuro toxic by-product of the processes was determined and served as a critical index for evaluation of the process and product quality. Limitation of jaggery compared to sucrose with respect to product specification is reported.

Keywords : Inversion, Enzyme, Jaggery, Sucrose, Hydroxy-methyl-furfural, Immobilization.

Invert sugar is a valuable sweetener in food and pharmaceutical applications. A process for inversion using enzyme has been transferred to industries for commercialisation by Food Technology Division of Bhabha Atomic Research Centre. The process is based on whole cell immobilisation of yeast cell, tailor-made to obtain maximum bio-catalytical effect (Ghosh and Nerkar 1991). The product is available in the market and finding new areas of applications e.g., tobacco, shoe polish, face cream etc.

Sugar (sucrose) is used as a raw material in the process. However, for the reason of easy availability and low cost, the possibility of using jaggery as a substitute to sugar looks attractive in both enzyme and chemical catalysed reactions. The present communication is aimed to analyse the issue pertaining to the suitability of jaggery obtained from the processing of sugarcane over sugar as raw material for inversion process.

Jaggery and sugar were purchased locally. Solutions of jaggery/sugar (60%, w/w) in distilled water was prepared and the acid (HCl) catalysed inversion was studied at pH 1-5 at temperature range of 50-70°C up to 8 h of time in a beaker kept over a thermostat controlled magnetic stirrer. Enzymatic inversion was performed at pHs 3 and 5 at temperature of 50°C for a period of 8 h using 7% (w/v) immobilised invertase (Ghosh 1994) in a beaker containing jaggery/sugar solution.

Glucose and fructose were estimated as total reducing sugars by the dinitrosalicylate method of Miller (1959). Hydroxymethylfurfural (AOAC 1990) and colour (ISI 1980) were estimated by spectrophotometric method.

Acid hydrolysed inversion of sugar and jaggery with time at temperature range (50-70°C) and pH (1-3) are depicted in Fig. 1 and 2, respectively.

At pH 1 and 50°C, the times required for complete inversion of sugar and jaggery were 6 and 2 h, respectively. As the temperature was increased to 60°C, the time for complete inversion was reduced to 2 and 1 h for sugar and jaggery, respectively. Further increase in temperature resulted in lesser reduction in time for 100% inversion as seen from Fig. 1 and 2. Similarly, at pHs 2 and 3, the percentage of inversion increased as the temperature was increased from 60 to 70°C. Shift in pH from lower to higher scale resulted in reduced inversion at a fixed temperature, confirming that inversion rate was dependent on increase in temperature and lowering of pH (Kirk and Othmer 1954). At 50°C, there was no inversion at pH 3 and above. The present study also revealed no inversion of sugar and jaggery at pHs 4 and 5 at the applied range of temperatures.

However, the said inversion was achieved by enzymatic action of invertase on sugar and jaggery at pH 3 and above and at 50°C. The enzymatic inversion of jaggery and sugar with respect to time at 50°C and pHs 3 and 5 is shown in Fig.3. It was observed that 50 and 80% inversion resulted in case of sugar and jaggery at pH 3 and 50°C, while the percentage conversion for the same at pH 5 was 45 and 79%, respectively. Immobilised enzyme was used in the form of particles in the

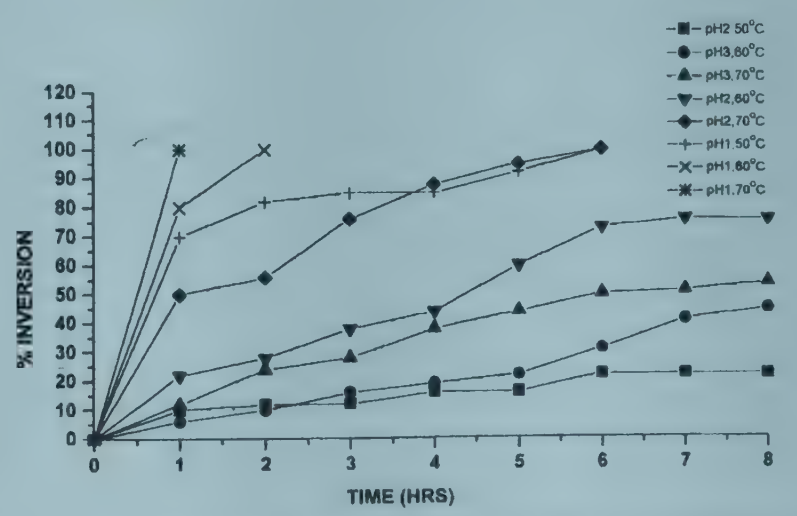

Fig. 1. Non-enzymatic inversion of sugar with time at different pHs and temperatures

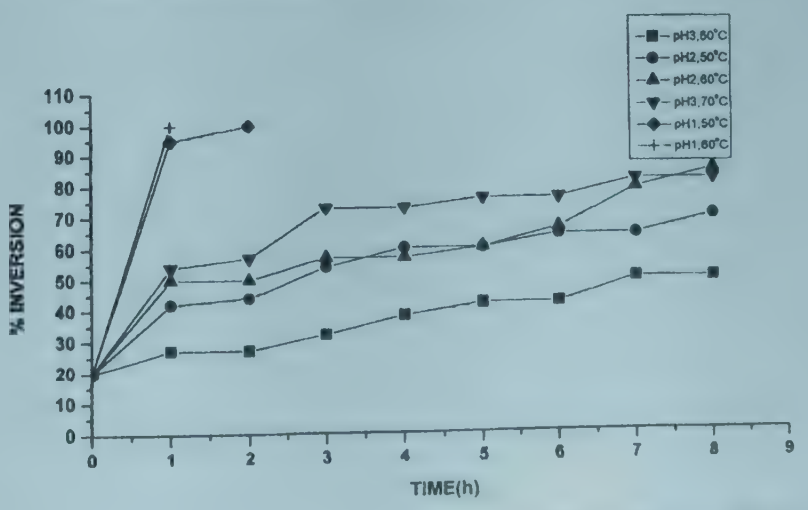

Fig. 2. Non-enzymatic inversion of jaggery with heating at different pHs and temperatures

* *Corresponding Author*

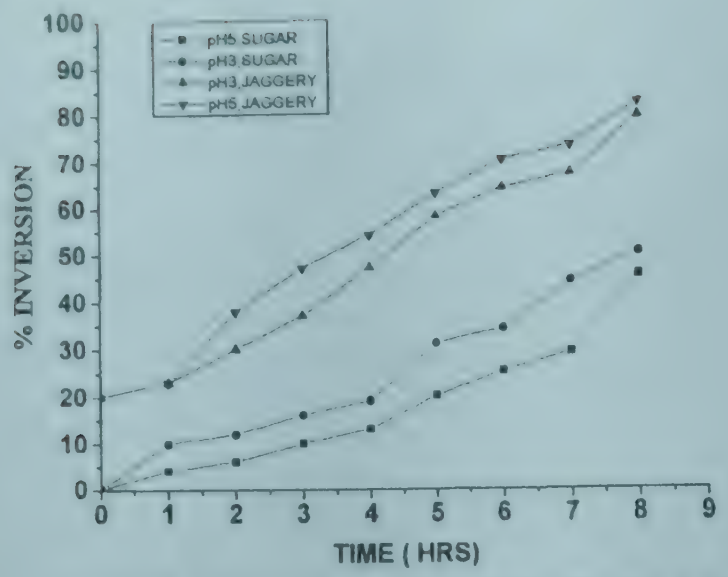

Fig. 3. Enzymatic inversion of sugar and jaggery with time at pHs 3 and 5 and 50°C

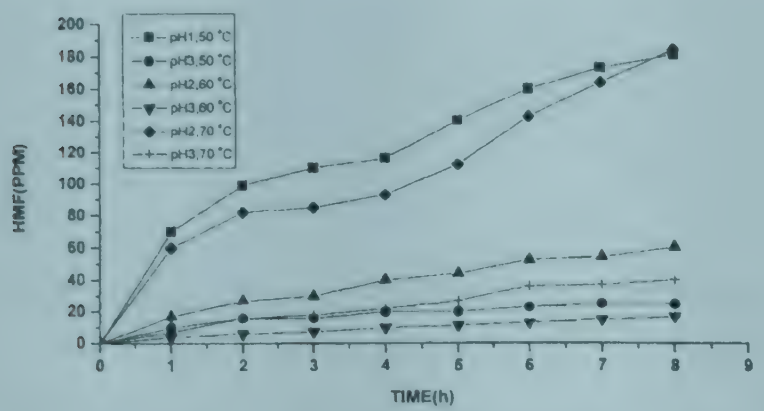

Fig. 4a. Concentration of HMF in non-enzymatically inverted sugar solution with heating at different pHs and temperatures

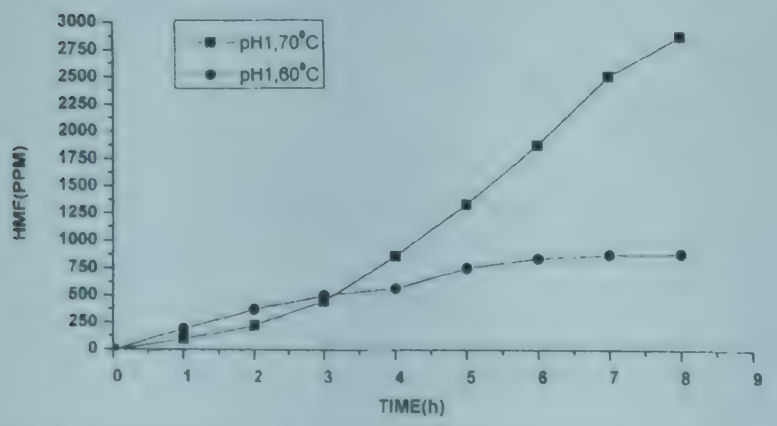

Fig 4b. Concentration of HMF in non-enzymatically inverted sugar solution with heating at pH 1 and 60°C, 70°C

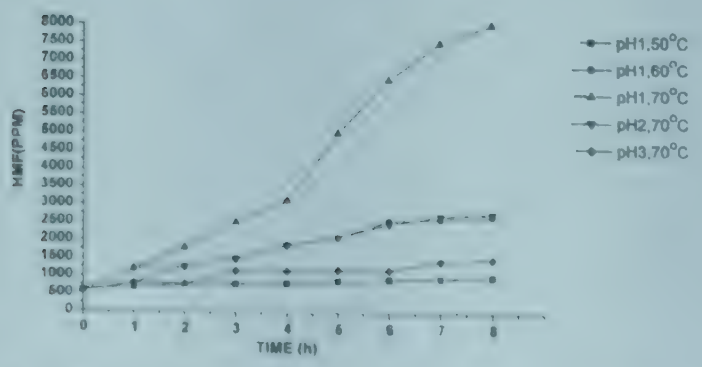

Fig. 5 Concentration of HMF in non-enzymatically inverted jaggery solution with heating at different pHs and temperatures

diameter range of 2 to 5 mm. The corresponding activities were 301 and 265 IU, respectively The optimum temperature of the enzyme was 60°C and it had a broader pH range (Ghosh 1994). However, for better operational stability, the temperature optimum selected was 50°C and pH above 3 (as per food chemical codex).

Hydroxymethylfurfural is one of the main by-products of the acid hydrolysed reaction. It is irritant to mucus membrane and is a poison to central nervous system (Parmeggini 1983). The maximum permissible limit of HMF is 80 ppm. It is, therefore, essential to estimate the quantity of HMF in the product. Fig. 4a, 4b and 5 represent HMF formation for acid hydrolysed reaction with respect to time at employed pH and temperature range for sugar and jaggery, respectively. Commercial sugar does not contain any HMF, but, there are wide variations in the HMF contents of jaggery, ranging from 50-600 ppm. Experiment was carried out with jaggery having initial HMF content of 580 ppm. At pH 1 and 50°C, the maximum HMFs formed at the end of 8 h were 190 and 1000 ppm for sugar and jaggery solutions, respectively. Increase in the temperature to 60°C, resulted in formation of HMF by almost 4.5 and 2.7 times more for sugar and jaggery, respectively. Further increase in the temperature to 70°C, 14 times i.e., 2800 ppm for sugar and 8 times i.e., 8000 ppm for jaggery increase in HMF values were observed.

However, at pH 2 with increase in temperature, there were increases in HMF values with time at a rate lower than the rate observed in case of sugar at pH 1. In the case of jaggery, at pH 2, the change in HMF was observed only at 70°C and the maximum HMF formed was 2750 ppm. For sugar, at pH 3 and the temperatures of 60 and 70°C, maximum HMF values observed at the end of 8 h were 15 and 40 ppm, respectively. There was no formation of HMF at pH 3 and 50°C in the absence of inversion. For jaggery, at this pH, the change in HMF value was observed at 70°C and the maximum HMF recorded was 1500 ppm. At pH 4 and 5 as well as the employed range of temperatures, no changes in HMF value were observed for both sugar and jaggery.

Hydroxymethylfurfural formation also depends on the pH of the experimental conditions. For sugar and jaggery, at 50°C, shifting of the pH from 4 to 3, the rate of HMF formation was 2.5 and 115 ppm per h, which was changed to 13.7 and 150 ppm per h, respectively, when the pH was shifted from 3 to 2. Still higher rate of HMF formation of the order of 326 and 725 ppm per h was recorded for sugar and jaggery, respectively, on shifting pH from 2 to 1 at the end of 8 h. For both sugar and jaggery, the maximum HMF formation occurred, when the pH was changed from 2 to 1 and the temperatures shifted from 60 to 70°C.

This study confirmed that at pH 3 and below, the maximum HMF formation occurred and the rate of HMF formation was enhanced with increase in temperature, which confirmed the observations of Singh and Kaur (1998).

Fig. 6 represents HMF formation with time for enzymatic reaction. The maximum amount of HMF formed at pH 3 was 2.5 ppm and there was no detectable amount at pH 5. There

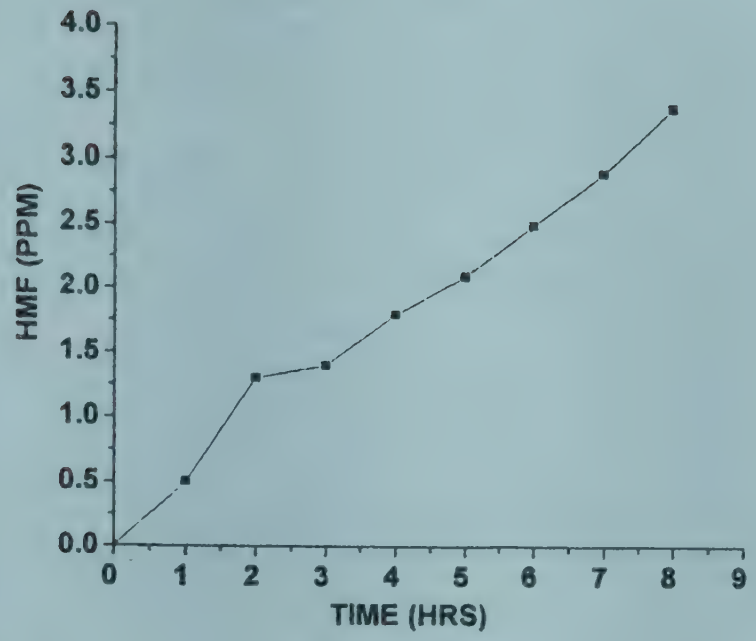

Fig. 6. Concentration of HMF in enzymatically inverted sugar solution with heating at pH 3 and 50°C

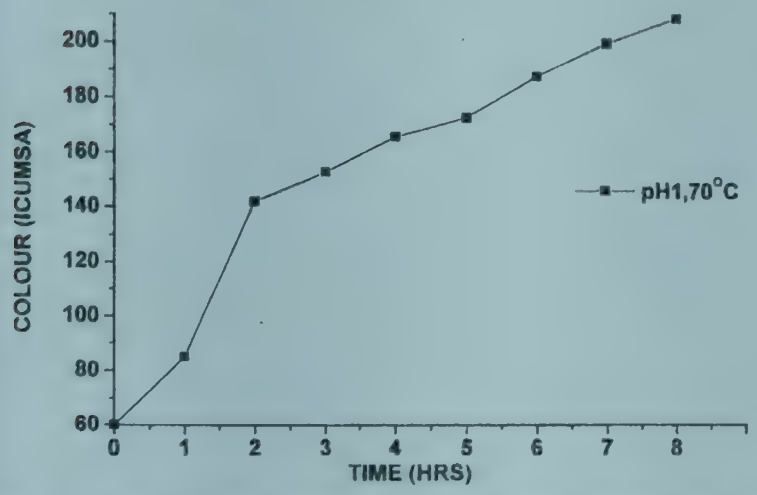

Fig. 7. Colour of non-enzymatic inverted sugar solution with heating at pH 1 and 70°C

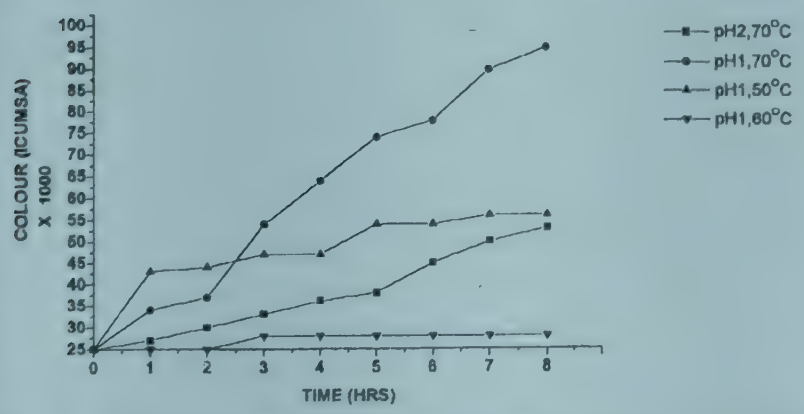

Fig. 8. Colour of non-enzymatic inverted jaggery solution with heating at different pHs and temperatures

was no change in initial value of 580 ppm for jagery after inversion under the experimental condition.

Colour plays an important role in food and pharmaceutical applications. While specification for soft drinks is prescribed strictly below 30 ICUMSA, no specification for confectionery industries exists and the requirement for pharma industries is specificied to be below 50 ICUMSA. The available BP standard, however, specifies colour as golden yellow to brown colour, corresponding to 100 to 150 ICUMSA. The initial value of colour

TABLE 1. SPECIFICATION FOR INVERT SUGAR

Taste and odour	No molasses, metallic or other foreign taste and odour
Colour	White to golden yellow (max. 150 ICUMSA)
Inversion	97% and above
Glucose : Fructose	1:1
pH	4.5 to 6.5
Ash	0.15 max
HMF	80 ppm max

of sugar used in the experiments was 60 ICUMSA. For sugar, no detectable change in colour was observed under the employed set of conditions except at pH 1 and 70°C wherein, the colour of sugar solution changed, reaching a value of 208 ICMUSA at the end of 8 h (Fig.7). Initial colour of jaggery solution was 25000 ICMUSA. At pH 1, the colour indices of solutions were 28000, 56000 and 90000 ICMUSA, after 8 h of heating at 50, 60 and 70°C, respectively (Fig. 8).

The recommended pH for invert sugar as per food chemical codex and BP standard is above pH 3 and preferably in the range of 4.5-5.5. Although at and below pH 2, the rate of inversion was higher at any fixed temperature, the inversion at this pH had not been recommended in view of stipulated specifications and formation of higher HMF. At pHs 4 and 5, there was no acid hydrolysed inversion, and hence, it was not suitable for jaggery inversion. Possibility of inversion below pH 2 and then readjusting the pH to 5 and above could be thought of. However, this will lead to high ash formation and deviation from specification (Table 1).

Inverted sample on heating at temperature above 70°C resulted in the formation of a dark coloured product and higher amount of HMF. The rate of HMF formation at temperature ranging from 28 to 120°C was studied and found to be 0.225 ppm/°C/h for the temperature range of 28-90°C and for the temperature range of 90-120°C, it was 10 ppm/°C/h. Therefore, the use of invert sugar with initial high contents of HMF for any food preparation involving high temperature processing would result in further accumulation of HMF in the final product. This should be avoided for the safety of consumers.

It was observed that when there was no conversion by non-enzymatic method, significant rate of inversion was achieved by enzymatic method under the employed set of experimental conditions. Enzymatic inversion of sugar resulted in invert sugar, conforming to the specification. Inversion by both enzymatic and non-enzymatic methods for jaggery resulted in the formation of higher quantity of neuro toxic HMF beyond the recommended limit. Colour of inverted jaggery was beyond the limit of any specification. It also defied the taste and odour specification of invert sugar (Table 1) by imparting the metallic odour. Moreover, SO_2 content of jaggery was almost 6-7 times more than the recommended value of 70 ppm (Patil and Adsule 1998). In view of all these facts, use of jaggery as a raw material in inversion process may be avoided.

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RESEARCH NOTE

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Solar Air Heating Module for Disinfestation of Foodgrains

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A simple flat plate solar air heating module using cheap materials like thermocol and aluminium sheet was developed for insect disinfestation of foodgrains, as this could be considered as a novel approach for preserving food materials, especially in developing countries like India, where plenty of sunshine is available throughout the year. During the sunny days, air temperature of 65°C-75°C, which is sufficient to kill all insect stages can be obtained by keeping the device on the terrace of the experimental station. The utility of the solar device for insect disinfestation of foodgrains was demonstrated by exposing artificially infested mung bean grains for a period of 90 min. The exposure time was found to be sufficient to kill all the stages of *Callosobruchus maculatus* feeding inside the mung bean grains. The exposure of the seeds to this temperature for given experimental time had no effect on germination.

Keywords : Solar air heater, Insect disinfestation, Bruchids, *Callosobruchus maculatus*, Mung beans.

Insect damage to stored cereals and pulses has been of great concern to human beings throughout the ages. The economic losses due to insect damage to stored grains are alarming. Insect infestation not only causes loss in quantity but also reduce the quality by webbing, adding fifth and off odours. Further, nutritive value of infested grain is adversely affected due to decrease in B-vitamins, essential amino acids and protein efficiency ratio (Swaminathan 1977).

Various methods of insect control have been practised with varying degrees of success, but the problem of cost and possible adverse effect upon the product limit their use. Fumigation though by far the most effective method of grain disinfestation, has its serious limitations. Many countries have banned the use of ethylene dibromide and ethylene oxide on account of their toxic residues in foods. Methyl bromide is to be phased out by the year 2000 under the Montreal Protocol as substances that deplete the ozone layer. There are some reports that insects developed resistance to phosphine. Ionising radiation treatment (0.25 to 1.0 kGy) can be used for control of infestations (Thomas 1998). The use of irradiation is promising but needs costly infrastructure for commercialisation.

In India, tropical weather is very conducive for insect development. Most of the farmers in our country neither have access nor can afford to use fumigants. Therefore, an alternative cheap method of disinfestation by solar heat will be of great help. All insects are susceptible to killing by heat (Nakayama et al. 1983). Dermandt and Evans (1978) used fluidized-bed heating for grains disinfestation. Solar heat has been suggested as means to eliminate bostrychid infestation in maize (Mc Farlane 1989). Beetles of the family Bruchidae are the most important pests of whole stored pulses. Among these, *Callosobruchus maculatus* F., is the most serious pest of stored pulses throughout the world (Jakai and Daoust 1986). In the present communication, a simple solar air heating module for disinfestation of pulse grains without affecting their germination qualities is described. The efficacy of the solar heating device was examined by artificially infesting mung bean (*Vigna radiata*) grains with different developmental stages of *C. maculatus*.

Construction of solar air heating module : A flat plate solar heating device (60 x 30 x 45 cm) was constructed from a thermocol box (Plate 1). It had two compartments (Fig. 1). The lower compartment A (60 x 30 x 30 cm) used for keeping seeds for treatment had a roof of flat plate absorber C. The absorber was made up of a aluminium sheet of 1.6 mm thickness measuring 58.6 x 46.3 cm in size. It was coated with black paint on the exposed side. A slit D was made in the absorber to allow circulation of hot air between two chambers. The hot air was circulated by a small blower E fixed at the absorber itself. A small flap F was fixed over the slit to direct the air on plate absorber. The chamber B was collector with size of 60 x 30 x 7.5 cm with glass (3 mm thickness) roof to seal this chamber. This solar heater module was inclined at a pre-determined angle to the horizontal, depending upon the month of the year such that at solar noon, the solar radiation was normal to the absorber plane to maximise the capture of solar insolation by the absorber.

Recording of air temperature in the solar module : The temperature of the inside air attained during the different time

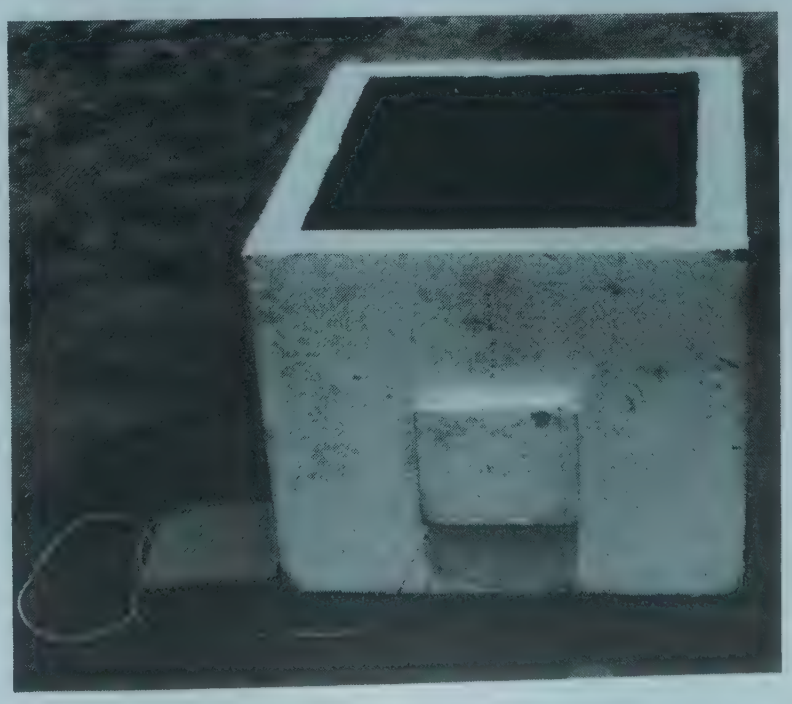

Plate 1. A photograph of solar air heating module

* *Corresponding Author*

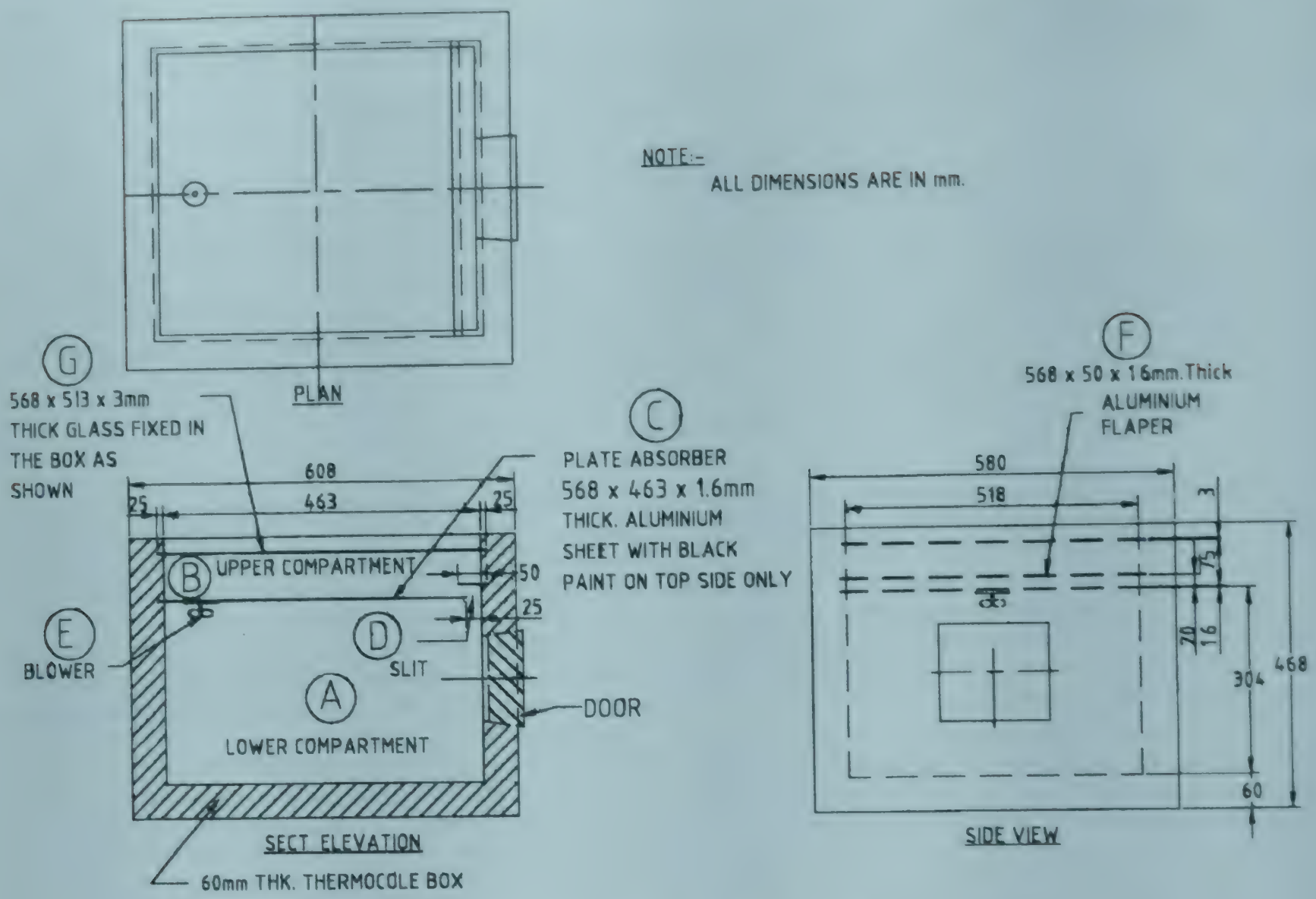

Fig. 1. Drawings of solar air heating module

period of the day were recorded, using a digital thermometer. Temperatures were recorded in the month of January to March and September to December.

Artificial infestation and method of disinfestation in solar air heating module : Insects used in these experiments were drawn from laboratory colony of *C. maculatus*, maintained on mung bean (*Vigna radiata*) seeds at 29 ± 1°C and 60 - 70% RH. One kg of mung bean grains were packed in bags made of coarse cloth. Fifty pairs of adults were released in each bag and closed tightly with a string. To carry out the disinfestation experiments, artificially infested one kg mung beans were exposed to hot air at temperatures between 65 and 75°C in the solar module for 30, 60 and 90 min. Different lots of infested bags were exposed to heat treatment after 0, 3, and 14 days after infestation, which represented adult, egg, and larval stages of insect development. Each treatment was replicated for five times and treated grains were held at 29°C. After 3 months of storage, percent grain damage was scored on the basis of samples drawn from each bag. Five samples of 100 grains were drawn from each bag and scored for insect adult emergence holes. Unheated grains served as control.

Seed germination : Randomly selected samples from control as well as heat treated lots were subjected to germination tests. Samples of 10 seeds were kept in flat petri plates (9 cm diameter) with moist filter paper. Total five replications were kept for control and treated bags. Seed samples were observed daily for their germination for a period of one week.

Air temperatures attained in solar module : The performance of the solar device was evaluated by recording air temperatures attained during time of the day. Data on the day temperatures attained in the month of January, February and March are shown in Fig. 2. The maximum air temperature of 75°C was recorded between 1 p.m. and 2 p.m. The air attained the temperature of 65°C by 11 a.m. and remained around 70°C at 3.00 p.m. Similar temperatures with little variations were attained during other months from September to December.

Disinfestation of artificially infested mung bean grains : Mung bean grains were exposed to hot air in the solar module for 30, 60 and 90 min. The percent grains damaged by *C. maculatus* after three months of storage are given in Table 1. No grain damage was observed, when mung beans infested with egg, larva and adult stages were exposed for 90 min to hot air with temperature of 65°C and above. Exposure for 30 min was found to be ineffective, as 34, 52, and 64% grain were found to be damaged, when grains infested with egg, larva and adult stages respectively. When grains were infested with adult

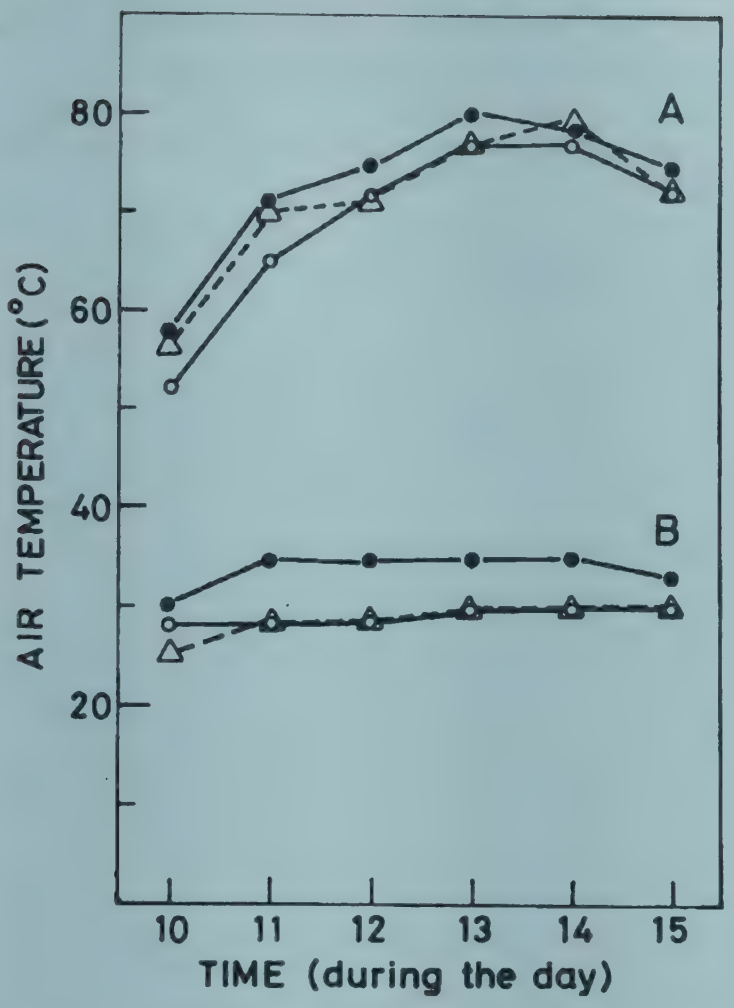

Fig. 2. Temperature of the air during the day
(A) Inside the solar heating module
(B) Outside the solar heating module
January - O - February - Δ - March - ● -

stage, percent grain damaged at this exposure of 30 min was not statistically different from the grain damaged in control, whereas, in case of other stages i.e. grains infested with egg and larval stage, percent grain damaged at exposure of 30 min was significantly different from the percent grains damaged in unexposed grain. No grain damage was observed, when grains infested with egg stage were exposed for 60 min, while 54 % damage was observed in grain infested with adult insects. A 68% damage was observed in control. The exposure time of 90 min was thus found to be sufficient to eliminate all the stages of insects attacking mung bean grains. The experiments were repeated with artificially infested mung bean grains with different stages of insect and exposure time of 90 min. At this exposure, there was 100% kill, when grains were infested with adult insects and there was no emergence from grains infested with other stages of insect development. Thus, these studies clearly established the efficacy of solar air heating module in eliminating all stages of insect development such as egg, larva, pupa and adult from mung bean grains.

Germination of mung bean seeds : No difference in % germination was observed between the controls and heated mung bean samples. Further, when these seeds were sown in the field, no adverse effect of heat treatment on seed germination as well as on plants growth was observed.

The present results showed that by using a simple solar heating module, air temperatures between 65-75°C can be

TABLE 1. PERCENT GRAINS DAMAGED AFTER 3 MONTHS OF STORAGE WHEN MUNG BEAN GRAINS INFESTED WITH VARIOUS STAGES OF *C. MACULATUS* WERE TREATED IN SOLAR AIR HEATING MODULE

Time of exposure, min	Percent grains damaged when grains were infested with Egg Stage (3 days postlaying)	Larval Stage (14 days postlaying)	Adult Stage (0 day)
0 (Control)	67.58[a]	67.58[a]	67.58[a]
30	34.32[b]	51.96[b]	63.91[a]
60	0.0[c]	0.80[c]	53.63[b]
90	0.0	0.0	0.0[c]

Means within the column followed by the same letter are not significantly different at 5% level of probability (Duncan's multiple range test)

obtained, which is 40-45°C above ambient temperature. This rise in temperature is much higher as compared to 20-25°C reported for other similar solar dryers (Selcuk 1971). This could be due to hot air being re-circulated in our system, while in other dryers, it was exhausted to the atmosphere. The exposure of grains to the air temperature of 65°C for 90 min was sufficient for effective disinfestation. All stages of *C. maculatus* infesting mung bean grains were killed by exposing them to this temperature. The present results are similar to Murdock and Shade (1991), who reported similar lethal temperatures for cowpea weevils. Disinfestation by solar heat has an edge over radiation disinfestation, as in the later case, very high dose is required to kill adult insect and low doses induced infertility (Dongre et al. 1997). The construction of solar air heating module is very simple. The materials needed are inexpensive and widely available. It is envisioned that the technology can be applied by the farmer at the farm itself at the time of harvest. The present results also showed that exposure of mung seeds to a temperature of 65°C for 90 min had no effect on germination and was suitable for sowing as confirmed by field experiments. Similar observations were made by Murdock and Shade (1991) with cowpea. Thus, these studies clearly established the use of solar heat for disinfestation without any adverse effects on seeds, which can be used for consumption or for sowing. The capacity of this small module for disinfestation was 30 kg/day. The present solar heater is conceptual in nature and has established its efficacy for disinfestation of foodgrains. To achieve optimum performance, the efficiency of energy collection, the capture of solar insulation by the absorber and heat transfer to pulses can be maximised by further experimentation.

Authors thank Mr. S.V. Pawar for the photograph and Mr. K.B. Patil and Mr. D.D. Shinde for drawings and fabrications.

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RESEARCH NOTE

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Effect of Heat Treatment and Soaking on Polyphenols of Redgram (*Cajanus Cajan*, L.)

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Polyphenolic contents of six varieties of redgram were determined after subjecting them to dry and moist heat, cooking in open pan and soaking overnight in water. All the treatments resulted in lowering the polyphenolic contents.

Keywords : Redgram, Heating, Cooking, Soaking, Polyphenols.

Redgram also called as pigeonpea (*Cajanus cajan,* L.) has occupied an important place in human nutrition as a rich source of proteins in diets of majority of population in India. It is a valuable source of minerals and vitamins and occupies a very important place in human nutrition in many developing countries. However, it is known to contain many antinutritional factors including polyphenols, which are generally located in the seed coat of pigmented cultivars of redgram (Singh 1988) and are known to reduce the activity of digestive enzymes (Singh 1984). Other antinutritional effects, which have been attributed to these polythenols include damage to the intestinal tract, lowered feed efficiency and growth depression in animals (Reddy et al (1985). Redgram is consumed by people in various forms. In India, it is processed into *dhal,* which is consumed after cooking to a desirable softness, but in some African countries, whole redgram seeds are consumed after boiling. Cooking not only improves the palatibility of redgram but also destroys or minimises some antinutritional factors (Singh 1988). Soaking usually forms an integral part of the processing method. It involves convenience and saves fuels, as it facilitates cooking. The present investigation was aimed to study the effect of heat treatment and soaking on polyphenols of redgram.

Six varieties of redgram viz. 'GC-7-133-1', 'GPC-PDIX-BDM-1', 'DMPT-GC-7-133-1', 'DMPT-GC-94', 'F2-perrinial-ICPL-63' and 'GPC-ICPL-87067' were procured from Pulse Research Station, Gulbarga. All chemicals used were of analytical grade. These were subjected to the following heat treatments.

Dry heat : The raw seeds and the overnight soaked seeds were heated at 100°C for one hour in a hot air oven. The heated seeds were then powdered and assayed for polyphenolic content by the method of Folin and Denis (1915).

Moist heat : The raw seeds and those soaked overnight were autoclaved at 15 Lb/inch at 121°C for 10 min. They were powdered and then, assayed for polyphenolic content by the same method.

Cooking : The raw seeds and the soaked ones were cooked for various time intervals and then assayed for polyphenolic content as above.

Soaking : Redgram seeds were soaked overnight in water at 4°C and water was discarded the following morning. The seeds were then dried with filter paper and assayed for polyphenolic content.

Table 1 shows the data on the effect of dry heat treatment on polyphenolic contents of raw and soaked seeds of redgram. Polyphenols were found to be reduced, when the seeds were subjected to dry heat, the degree of reduction being more in soaked seeds than the raw ones. These results are in agreement with Baber et al (1988), who reported reduction in polyphenols of jackbean as a result of heating at 100°C for 60 min.

The data on the effect of moist heat on polyphenolic content of raw and soaked seeds of redgram are presented in Table 2. A decrease was observed as a result of autoclaving and there was more decrease in soaked seeds than the raw ones. These observations are in conformity with those reported by Expenyoung (1985), who reported that autoclaving of both cereals and legumes resulted in larger losses of polyphenolic compounds. He reported that the large loss may be attributed

TABLE 1. EFFECT OF DRY HEAT ON POLYPHENOLS OF REDGRAM (*CAJANUS CAJAN*,L.)

Name of the variety	Polyphenols, mg/g Untreated ± 1 SD seeds	Raw ± 1 SD seeds	Soaked ± 1 SD seeds
'GC-7-133-1'	1.80 ± 1.40	2.00 ± 1.70	1.50 ± 1.15
'GPC-PD1X-BDM-1'	1.65 ± 0.60	3.00 ± 1.45	2.80 ± 1.20
'DMPT-GC-7-133-1'	2.70 ± 1.90	2.50 ± 1.85	1.00 ± 0.50
'DMPT-GC-94'	2.60 ± 1.50	1.20 ± 0.40	1.00 ± 0.27
'F2-Perrinial-ICPL-63'	2.00 ± 1.02	0.85 ± 0.46	1.00 ± 0.22
'GPC-ICPL-87067'	1.22 ± 0.60	2.00 ± 0.62	1.20 ± 0.25

Each value is an average of triplicate determination

TABLE 2. EFFECT OF MOIST HEAT ON POLYPHENOLS OF REDGRAM (*CAJANUS CAJAN*,L.)

Name of the variety	Polyphenols, mg/g Untreated ± 1 SD seeds	Raw ± 1 SD seeds	Soaked ± 1 SD seeds
'GC-7-133-1'	1.80 ± 1.40	1.35 ± 0.20	0.60 ± 0.70
'GPC-PD1X-BDM-1'	1.65 ± 0.60	1.35 ± 0.60	0.67 ± 0.70
'DMPT-GC-7-133-1'	2.70 ± 1.90	1.70 ± 0.37	0.97 ± 0.50
'DMPT-GC-94'	2.60 ± 1.50	1.02 ± 0.40	1.00 ± 0.50
'F2-Perrinial-ICPL-63'	2.00 ± 1.02	0.95 ± 0.40	2.00 ± 0.47
'GPC-ICPL-87067'	1.22 ± 0.60	1.07 ± 0.60	2.00 ± 0.32

Each value is an average of triplicate determination

* *Corresponding Author*

TABLE 3. EFFECT OF COOKING ON POLYPHENOLS OF REDGRAM (*CAJANUS CAJAN*,L.)

Time, min	'DMPT-GC-7-133-1' ± 1 SD		'DMPT-GC-94' ± 1 SD	
	Cooking	Soaking and cooking	Cooking	Soaking and cooking
Untreated	2.70 ± 1.9	2.70 ± 1.9	1.50 ± 2.6	1.50 ± 2.6
10	2.50 ± 0.3	0.87 ± 0.4	1.02 ± 0.2	0.70 ± 0.5
20	2.20 ± 0.7	0.77 ± 2.0	0.87 ± 2.0	0.35 ± 0.5
30	1.85 ± 2.4	0.72 ± 2.0	0.72 ± 2.5	0.20 ± 0.1
40	1.25 ± 1.0	0.40 ± 4.5	0.42 ± 0.2	ND
50	1.10 ± 0.6	0.25 ± 2.5	0.42 ± 0.1	ND
60	0.57 ± 0.5	ND	0.27 ± 0.3	ND
90	0.35 ± 1.2	ND	ND	ND
120	ND	ND	ND	ND

Each value is an average of triplicate determination, ND-Not detected

TABLE 4. EFFECT OF SOAKING ON POLYPHENOLS OF REDGRAM (*CAJANUS CAJAN*,L.)

Time, h	'DMPT-GC-7-133-1' ± 1 SD	'DMPT-GC-94' ± 1 SD
Untreated	2.70 ± 1.90	1.50 ± 0.60
Soaked in distilled water		
6	2.10 ± 0.30	1.35 ± 0.24
12	2.20 ± 0.75	0.70 ± 0.90
18	1.85 ± 0.60	0.60 ± 0.20

Each value is an average of triplicate determination, ND-Not detected

to two possibilities. (i) the polyphenols are destroyed during the traditional cooking, which is similar to moist heating and (ii) it is possible that the polyphenols form complexes with other water soluble substances and get discarded with the cooking broth. This loss might be due to the fact that phenolic hydrogen bonds between the hydroxyl group in the phenolics and their receptor groups bind together forming protein phenolic complexes. Kaur and Kapoor (1990) also reported maximum reduction of polyphenols, when the soaked seeds of rice bean were subjected to autoclaving.

Data on the effect of cooking on polyphenols of redgram are given in Table 3. A gradual decrease in polyphenols was observed, when the cooking time was increased from 10 min to 120 min. The decrease was more, when the seeds were soaked prior to cooking. These observations are in agreement with Baber et al (1988), who reported that when the dry seeds of jackbean were cooked for 60 min, there was 60% reduction in polyphenolic content. However, when the seeds were soaked for 24 h and cooked, almost all the polyphenols were lost within 25 to 30 min. Soaking as well as cooking resulted in losses of polyphenols due to leaching. It has been suggested that due to heat treatment, either solubility or chemical reactivity of polyphenols is changed, resulting in apparent decrease in assayable polyphenols (Butter et al. 1979).

Data on the effect of soaking redgram seeds in distilled water for 6, 12 and 18 h are given in Table 4. A gradual decrease was polyphenols is observed when redgram seeds were soaked in water. These results are supported by Rao and Deosthale (1982), who reported that nearly 50% of polyphenols were lost in *Bengal*gram and redgram as a result of overnight soaking in water. According to Mattson (1946), the loss of solids during soaking may be influenced by the concentration gradient (difference in chemical potential), which governs the rate of diffusion and by the physical barrier in cotyledon cellwalls, which limit the diffusion of water or the soaking media.

It may be concluded that heating, cooking and soaking, which are the most simple and inexpensive methods can be practised at household level to reduce the polyphenolic content of redgram.

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J. Food Sci. Technol., 2001, Vol. 38. No. 2, 189

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS : WHO Additive Series : 44 - Prepared by the Fifty Third Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) International Programme on Chemical Safety (IPCS) WHO, Geneva 2000 pp 534. Price Sw.fr.100/- Price in Developing Countries Sw. fr. 70/-

JEFCA in its several meetings before this (Listed in Annex 1) has been disseminating very important and useful information on toxicological studies on food additives and contaminants on the basis of biochemical aspects such as absorption, distribution in body tissues, excretion, effect on enzymes, reproduction studies, genotoxicity and teratogenicity in selected species - rats, mice, dogs, sheep, pigs and the like - both short and long term studies are included. This monograph gives results of such studies on several food additives and contaminants - some new studies and some further studies on distributional and dosage factors.

The first section deals with studies on a synthetic glazing agent - hydrogenated poly-1-decene, which has a well defined composition. Studies have shown that the ADI is not specified, until further studies are done later. Erithritol - a fermentation derived sweetener occur in wines, sake and soy sauce but also occurring naturally in fruits and mushrooms, was studied as above. Projected intakes vary from 1-5 g/day. Studies are done on various animals and some healthy volunteers at different dosage levels. Diarrhoea was noted in human volunteers at the maximum level. Human studies revealed that an intake of 1g/kg bw in a variety of foods for five days had no effect. However, ADI has not been specified. The No Observed Effect Level (NOEL) in animals was 1-2 g/kg bw.

Curdlan - a linear polymer of 1→3 linked glucose units is also a fermentation derived additive using the bacterium *Alcaligenes feacalis* var. *myxogenes*. Curdlan is used as a formulation and a processing aid, stabiliser, thickener and texturiser. Curdlan does not induce genotoxic, carcinogenic or teratological effects. The ADI has not been specified pending studies on usage levels in different foods and in different areas of the world.

Some miscellaneous substances like γ-cyclodextrin, which is 8 glucose 1→4 linked ring shaped molecule. This was previously evaluated at the 51st meeting of JECFA, without specifying ADI. Further studies revealed that human beings could tolerate concentrations of 8 g servings in different foods equivalent to 0.11 g/kg bw, but still, ADI is not specified. Sodium ion EDTA is used to fortify foods with iron, providing 0.2 mg/kg bw. Sodium sulphate was recently evaluated but this study also did not specify ADI, as the information on functional effect and actual uses in foods is not known.

A major part of the monograph deals with various types of Flavouring Agents. A scheme for safety evaluation of flavouring agents is very interesting as also the calculation of intake based on volume of production and population of consumers expressed as µg/person/day. About 137 flavouring agents that include simple aliphatic and aromatic thiols are listed according to names with structures and have been evaluated for safety. Also, 47 other flavouring agents that include aliphatic primary alcohols, aldehydes, carboxylic acids and esters have been evaluated.

Allergenicity of refined peanut and soybean oils have been evaluated for safety based biological data and studies on humans. Final safety evaluation needs further studies on the methods used for refining these oils.

The next study deals with toxic metal ions like lead and methyl mercury as well as a study on a non-steroidal mycotoxin zeralelone residues in cereals, legumes, milk, eggs, meat and wheat products. Lead, which was earlier evaluated, was given a provisional tolerable weekly intake (PTWI) of 50 µg/kg bw. Further studies dealt with countrywise findings based on soil types etc. Simulation models are used to evaluate effects of any planned interventions. Methyl mercury, also studied earlier, was assigned a PTWI of 300 µg of total mercury of 200 µg of methyl mercury per kg bw.

The next part of the study deals with food colours, natural and synthetic. Annatto seeds have been consumed by 44 million Brazilians as a condiment for many years at a level approx. 150% of ADI. Canthaxanthin has also been consumed above ADI levels. Erythrosine, earlier evaluated and given an ADI of 0-0.1 µg/kg bw was further studied countrywise. Iron oxides also earlier evaluated at 0-0.5µg/kg bw at the 23rd meeting of JEFCA was re-evaluated.

Annexe 4 summarises ADI values, toxicological evaluation and other pertinent data of all studies done and evaluated in this monograph. This is very useful. The monograph is recommended for use by government and regulatory authorities and food industry as well as anyone dealing in any aspect of food science and technology. The vast amount of data and methods used for safety evaluation of novel food additives and condiments is phenomenal. This monograph will serve as an eye-opener for all concerned.

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